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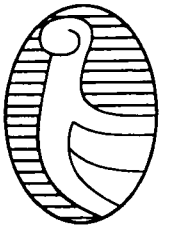

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SPATIAL SPREADING OF ROBINIA PSEUDO-ACACIA AND POPULUS ALBA CLONES IN SANDY HABITATS

V. Krízsik and L. Körmöczi

Krízsik, V. and Körmöczi, L. (2000): Spatial spreading of *Robinia pseudo-acacia* and *Populus alba* clones in sandy habitats. — *Tiscia 32, 3-8.*

Abstract. Invasion of alien species has become a general problem word-wide. Among the shrinking patches of natural vegetation, various human activities create artificial corridors for the migration of species which have been geographically separated, and have had distinct evolutionary histories. The time scale of invasions is much smaller than the evolutionary scale of the traits by which these species have been adapted to their environment. Black locust was introduced to the Hungarian Great Plain in the 19th century.
Its successful invasion nowadays seriously threatens the maintenance of natural communities over large areas, especially in sandy habitats. Our aim was to study the reasons for the rapid invasion of this species. In particular, we focused on its capacity for clonal reproduction.
We investigated the dynamics of ramet production and spatial spreading of *Robinia* clones in sandy habitats. In comparison, we measured the same traits in another clonal tree species, *Populus alba*. White poplar is an element of the natural vegetation in the same area.
Robinia proved to be more aggressive and successful in the occupation of vegetation gaps by strong establishment of brush-wood. Why is the Robinia so successful in the occupation of the sandy habitats of Great Hungarian Plain. In this respect, the ability and dynamics of clonal growth seems to be important. We hypothesise that the utilisation of vegetation gap differs in the case of a native and an adventive species

Keywords: growth strategy, competition, invasion, black locust, white poplar.

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Introduction

Degradation and disappearance of natural habitats has become a serious problem to nature conservation. Introduction of adventive plants is another critical phenomenon, because it threatens the integrity of our natural communities. This process is rather conspicuous in the Hungarian Great Plain. The explanation for the present alarming condition of its natural ecosystems over large areas goes back to decades in the history of land use (Hargitai 1940, Kovarik 1995b). Severe deforestation started in the 19th century due to demand for wood (Kertész 1984). As a result of cutting the natural forests, and simultaneously planting *Robinia pseudo-acacia*, some 80-85% of the forests of this region had become black locust stands by the beginning of the 20th century (Kiss 1922). The first appearance of *Robinia* in Europe owes to voyagers and horticulturists (Kovarik 1995a). *Robinia* came from the temperate zone of North-America (Kertész 1984), and it found suitable conditions for establishment in Europe. Its invasion has been helped by artificial plantations, but it is spontaneous at a coniderable extent. *Robinia* exemplifies those plants which have been introduced from other continents, and started their infamous carriers on the new land by uncontrolled expansion (see Pysek *et al.* 1995, Kovarik 1995a).

Another important factor that promotes unwanted invasion is the increasing size of agricultural and ruderal areas. Disturbed habitats provide suitable sites for the spreading of *Robinia*, and also for weeds, which have been evolutionarily adapted to the occupation newly opened vegetation gaps. The pressure from their seed rain and propagule bank has

also increased on the Great Plain (Halassy and Török 1996). A large set of ruderal species is presented in and around our natural habitats strongly connected to *Robinia*-woods. Therefore, their resistance to disturbance and invasion is a key issue which determines the future of our natural communities.

Continuous or regular disturbance is likely to destroy the natural structure of a community (see Margóczi, Körmöczi and Kincsek 1996). A threat in the expansion of *Robinia* forests is that they accumulate ruderal species in the understorey, therefore, they may become sources for future spreading of these species.

In this paper, we study the clonal expansion of *Robinia pseudo-acacia* stands. Clonality can be essential in reproducing and competing for resources in newly occupied areas (van Groenendaet *et al.* 1996). We investigate how clonality contributes to the successful establishment, growth and competitive dominance of black locust populations on the Great Plain.

Life-form, regeneration cycle and clonal growth habit strongly determine the population dynamics of *Robinia*, and the steps of its invasion. According to our preliminary assumptions, these traits lead to a specific spatial behaviour of this species, which may influence not only the structure of an existing stand, but also the subsequent regeneration of the natural vegetation in its site by secondary succession. Several authors have emphasised the role of clonality in foraging for resources, occupying new habitat patches, tolerance to environmental fluctuations, regeneration after adverse conditions, and defence against herbivores (Oborny and Bartha 1998, Herben *et al.* 1994, Sutherland 1990, van Groenendaet *et al.* 1996). Clonal growth rules can largely determine the long-term pattern of the occupation of habitat space (van Groenendaet *et al.* 1996, de Kroon and Schieving 1990).

The clonal growth form

A cause of the successful invasion of *Robinia* is probably its capability for quick spread by root-buds (Jenik 1994). It is very difficult to eradicate an established tree from the habitat. Developing sprouts, on one hand, is a strategy for growth and expansion, and on the other hand, an adaptive response for damage (Jenik 1994). This is why it is so difficult to clear black locust out.

Native communities on sand also contain a lot of clonal species. Clonality is frequent among herbs, grasses, shrubs and trees as well. Growth forms of the clones play very important roles in the structural development of vegetation. Growth dynamics of the species determine the pattern of ramets in space and the changes of this pattern in time (Oborny and Bartha 1998).

Those ramets that belong to the same genet and are connected by horizontal structures (such as rhizomes or stolons) are often capable of transporting nutrients, water and photoassimilates to each other. This is how mother ramets can subsidise their offspring until they become established, and thus, help to occupy new microhabitats and compete with neighbours. The clonal growth habit provides various opportunities for special resource exploitation strategies, which promote persistence in the sites that have been occupied. Thus, clonality can strongly influence the spatial pattern of plant communities, and their successional processes (Klimes and Klimesova 1994, Oborny and Bartha 1995).

The objective of our study on clonal trees

We hypothesise that clonality may be an important factor in the invasion of sandy habitats.

Therefore, we compare the success of a native and of an adventive, invasive species in the same site. The adventive species that we observe is *Robinia pseudo-acacia* L. The comparable native species is *Populus alba* L which is an edificator species of the *Junipero-Populetum albae* community (Simon 1979, Szodfridt 1969).

White poplar also shows predominance of clonal reproduction in its life history. Because of its economic use, this species has also gone through artificial selection, and several cultivated varieties exist.

The questions addressed:

1. What kind of spatial and temporal dynamics of clonal growth can be described/recognised in the case of *Robinia pseudo-acacia* and *Populus alba*?
2. What is the difference between the dynamics of clonal spreading in *Robinia pseudo-acacia* and *Populus alba*?
3. Could these traits be responsible for the difference in the success of the native and adventive species?

Material and methods

Study site

The investigations were carried out in the Kiskunság National Park, Great Hungarian Plain, near to villages Bugac and Fülöpháza.

This area is one of the typical sandy habitats with dry grasslands, *Festucion vaginatae* (Soó 1929, 1964-1980), and forest communities, *Junipero-Populetum albae* (Simon 1979, Szodfridt 1969, Körmöczi 1983, Fekete 1992). These communities form patchy vegetation, and provide *Robinia pseudo-acacia* suitable habitat. Their distribution is related to the sandy soil. The Bugac region is rather plain with slight undulation of the surface. In the Fülöpháza region, the elevation of sand-hills is more pronounced. The communities of the area have been formed due to the soil and climatic conditions, and have many centuries of history.

Their high biodiversity represents a great value for nature conservation. Grazing have been reported to control the expansion of white poplar and black locust patches (Kelemen *et al.* 1995). At the first sight, *Robinia* and *Populus* are rather similar in their ability to spread and attain local dominance. In Fülöpháza, for example, the numbers of *Populus* and *Robinia* patches are nearly equal. On the other hand, after a forest fire which happened at Bócsa in 1993, *Robinia* proved to be more aggressive and successful in the occupation of the newly opened sites by vigorous establishment of brush-wood.

The established locust stand then prevents the emergence of other woody species (Gobbi *et al.* 1995). Though neither kind of invasion plays a positive role in the vegetation diversity, but *Populus* is less detrimental in this region than *Robinia* because it is native and, as it can coexist with other characteristic, often valuable species of the sand flora.

The model plants

Individual genets were chosen for investigating the mechanism of clonal spread in sandy soil. We chose solitary mother trees with sprouts around them.

We measured the distance between the mother and the offspring ramets. We estimated the age of the mother and the new saplings from the diameter of the trunks at 1.3 m height. According to the literature, there is an allometric relationship between age and diameter (Sopp 1974). These relationships are frequently used in the practice of forestry. We used a linear model for estimating the age (determination coefficient>0.95; the standard error ranged from 0.16 to 2.43 years). The relationship depends on the quality of the environment, especially of the soil. Based on these differences, six quality classes are used in forestry. The origin of the tree is also important: sprouts differ from saplings that have derived from seeds.

The identification of these soil-based quality-classes is difficult, therefore we calculated the average of the six classes, and we used the data for the sprout-originated trees.

Because of the variety of factors that influence the age-diameter relationship, our estimation can be considered as a rough approximation.

We selected 13 white poplar and 14 black locust solitary genets at Bugac and Fülöpháza (about 12 km^2 each). We found and measured about 400 sprouts. After damage of the seminal shoot of the young trees (e.g. due to grazing), regeneration takes place with intensive branching, and both *Robinia* and *Populus* can take a bushy growth form. We considered those shoots that originate from one trunk as parts of one ramet, and they ramify above the ground. We infer to the spatial and temporal dynamics from the proliferation of the sprouts around the genet. We measured the distances of the offsprings from the mother tree, and calculated the age of them (from the diameter of the trunk). The age differences between the mother and their offsprings can be converted to time-delay by computing the years which passing till the appearance of a new offspring (relative time).

Results

The growth pattern in space and time

We used the spatial pattern of sprouts around each mother tree to reconstruct the temporal dynamics of clonal growth. We calculated the regression of the mother tree—sprout's distance in relation to their emergence time (the emergence time=mother age minus the age of the offsprings). We found a positive linear relationship between these variables. The correlation coefficient for black locust was r=0.52, and for white poplar somewhat weaker, r=0.428. The determination coefficient, however, was 0.27 for black locust, and 0.183 for poplar (Fig. 1). Although the correlations that we found were rather week, the points on the space-time diagram clearly show a non-random pattern.

What is the difference between *Robinia pseudo-acacia* and *Populus alba* in the dynamics of clonal spreading? Clear differences can be seen between the two clonal species in the distance and time of ramet emergence, calculated for all offspring ramets in the population (Figs 2 and 3). Poplar sprouts emerge later in time and further from the mother tree than black locust sprouts.

The distance of offsprings of *Populus* ranges from 1-2m to 10-20m from the mother. The area occupied by the clones of *Robinia* is smaller. It covers mostly less than 10m radius circle, and

generally the closest sprouts grow a few cm from the mother plant.

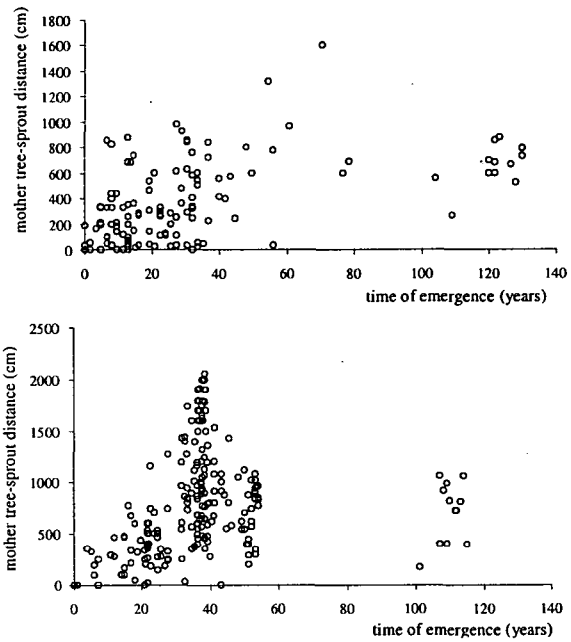

Fig. 1. Distribution of the distance of the ramets in relation to relative time of clone emergence. Upper: black locust (correlation 0.52, determination coefficient 0.27)., lower: white poplar. (correlation 0.43, determination coefficient 0.18).

The difference in the temporal pattern of clonal spreading is also remarkable. The reconstructed time course of sprouting shows that emergence of *Robinia* sprouts have shorter delay compared to the establishment of the mother tree. In general sprouting becomes less frequent with time. A sharp decrease is detected after 40 years in the frequency of emergence, in contrast *Populus* shows an increase in the frequency after 40 to 60 years.

Differences in space and time

Could the differences in the dynamics of expansion be responsible for the success of the adventive species relative to the native species?

The first twenty years proved to be decisive in the fate of the populations. *Robinia* has a definite advantage in the occupation of opened space: almost 50% of the sprouts of *Robinia* emerge in the first twenty years, but *Populus* develops only 4-5% of its ramets in this period.

Robinia has no such big difference in the intensity of sprouting in time. It produces similar proportion in the first and in the second twenty years.

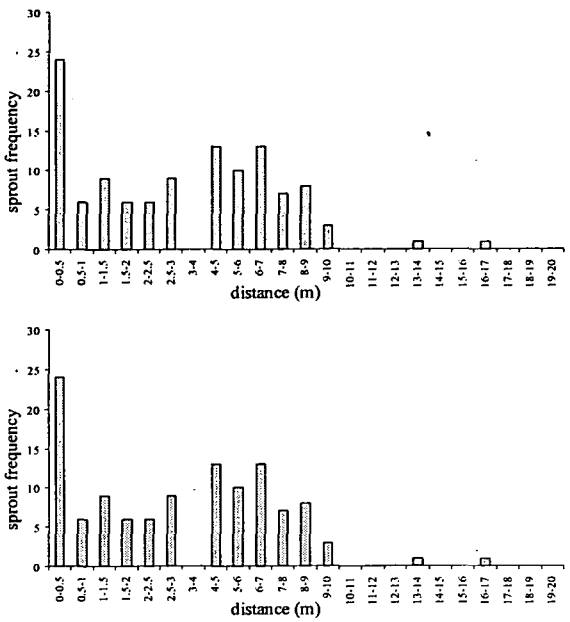

Fig. 2. Frequency distribution of the distance between mother and offspring (upper) and of the time of emergence (lower) in black locust.

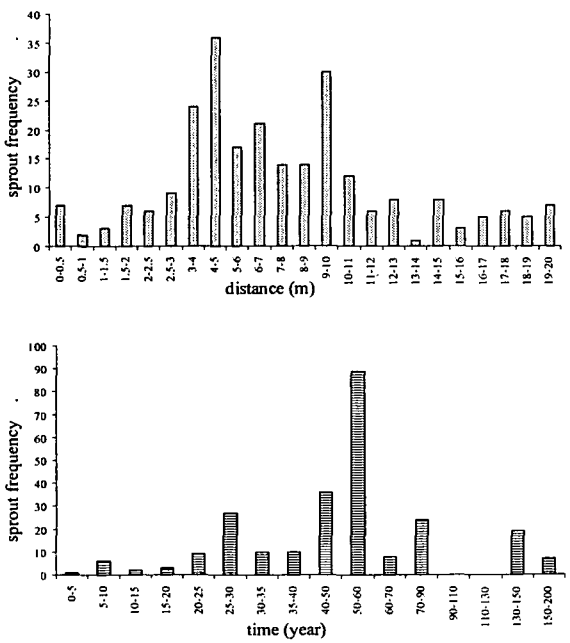

Fig. 3. Frequency distribution of the distance between mother and offspring (upper) and of the time of emergence (lower) in white poplar.

Populus shows an increase in the intensity of sprouting. In the first twenty years 4-5% of the sprouts emerged, and the rest was added in the second twenty years. No sprouting occurred from 60 to 100 years, and the frequency of later emergence was very low (Figs 2 and 3).

Growth dynamics of the individuals shows a different trend in the case of the two species. In the first twenty years, black locust shows quicker thickening than white poplar. But later, the diameter of poplar clones exceeds that of locust clones of the same age (Fig. 4).

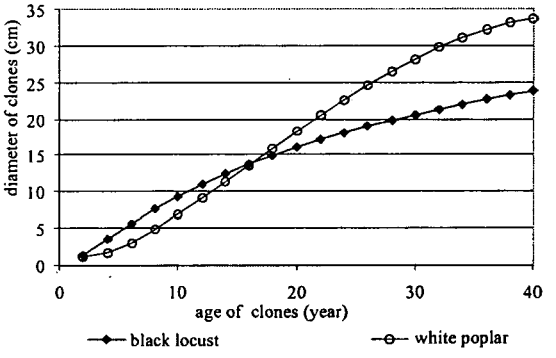

Fig. 4 Comparison of the growth dynamics of the two species (based on estimation from stand quality classes).

We used a linear model for age-estimation, but at a given age there are opposite deviations in the trunk's diameter of the two species, and the estimation of the ages may be false because of different growth dynamics. Therefore we checked the results by another age-calculation. We do not calculate the absolute ages, only the diameters of the trunks were compared. The diameter of the mother trunk was considered as 100%, and the percentage diameter of the sprouts was calculated compared to this. The difference of percentage diameter can be used as a relative time-delay. If the time-delay was estimated only from the thickness differences of the trunks, the same separation can be recognised between the black locust and the poplar sprout emergence.

Discussion

The dispersal pattern

The availability of propagules is very important in the occupation of gaps. An alternative way of spreading is clonal growth. An advantage of vegetative reproduction is that the mother plant can subsidise its offspring by nutrients, water and photo-assimilates. Thus, the vegetative offspring usually has much better chances for survival than the generative progeny (van Groenendaet *et al.* 1996). Vegetative spreading to new sites provides special opportunities for microhabitat selection (Herben *et al.* 1994, Oborny and Bartha 1995). Accordingly, clonal species play an important role in the succession of vegetation (Stöckling and Baumler 1998).

We reconstructed the dynamics of clonal growth on the basis of the spatial pattern and estimated age of sprouts around mother trees. Capacity for dispersal is very important in the exploitation of new openings in the habitat, therefore we investigated separately the spatial and the temporal pattern of spreading to draw the frequency distribution of sprout distances and of emergence time. The question is whether any deterministic rule can be recognised in the dynamics of clonal spread of *Robinia pseudo-acacia* or *Populus alba*. The first step is to recognise the process in space and in time, and find non-random patterns in the emergence of clonal offspring. As shown in Figs 2 and 3, several peaks can be found in the emergence frequency in space and time. The distribution of these peaks are different in the case of *Robinia* and *Populus*. *Robinia* sprouts emerge earlier and closer to the mother tree, while *Populus* have its offsprings further in space and later in time.

From the growth patterns detected in the present study, we can conclude that *Robinia* resembles more to the phalanx growth strategy, and *Populus* to the guerrilla strategy (Sutherland 1990).

We have presented some observable tendencies in the clonal growth habit of these species, but several additional factors may influence the growth pattern of a species in any particular habitat. For example, low availability of water and nutrients can significantly change the growth form, as well as herbivores or other injury in the root or shoot system (de Kroon *et al.* 1994). An interesting, community-level outlook from the description of growth strategies may be to study the „spatial combining" ability of different species (Herben 1995).

Conclusions

Robinia pseudo-acacia shows rather quick and aggressive lateral spreading compared to the native species, *Populus alba*. *Robinia* can vigorously invade the native communities on sand, and attains stabile persistence in the habitat as a dominant species. Why is *Populus* less successful in the invasion to new gaps and exclusion of other species?

One of the key factors may be that its clonal spreading is slower, and its stand of shoots is less tightly packed (more guerrilla-like).

Our study calls for further research on the role of clonality in sandy habitats, and on the dynamics of competition between native, stress-tolerant species and invasive clones.

Acknowledgements

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PHYTOSOCIOLOGICAL AND EDAPHYC ASPECTS OF THE INVASION BY *CLEISTOGENES SEROTINA* (L.) Keng IN THE KISKUNSÁG NATIONAL PARK

Cs. Szigetvári

Szigetvári, Cs. (2000): Phytosociological and edaphyc aspects of the invasion by *Cleistogenes serotina* (L.) Keng in the Kiskunság National Park. — *Tiscia 32, 9-17.*

Abstract. In the recent decades *Cleistogenes serotina*, a perennial grass native to Hungary has become a successful invader in the open sand grasslands of the Great Hungarian Plain. The aim of the present study is to detect if there is any relation between the different community types, soil properties and the dominance of the invader. Plant cover as well as seven soil parameters (soil water, organic matter and carbonate content, pH, nitrate, available potassium and phosphate concentration) were studied in a transect of 139 0.5×0.5 m quadrats on a slope of a sand hill in the Kiskunság National Park, Hungary.
Three distinct vegetation types were detected: *Cleistogenes*-dominated type, *Fumana procumbens*-dominated open perennial grassland type, and *Secale*-dominated open annual grassland type. The invader-dominated type was in many aspects — low cover of spring annuals and criptogams, high pH, low nutrient concentration — more similar to the *Fumana*-type (which is close to the original natural vegetation of the habitat) than to the *Secale*-type (which is considered a degraded vegetation type). In the *Cleistogenes*-dominated quadrats soil water content was higher than in the other two types, and a slight increment in the cover of the subordinate annuals was also detected as compared to the *Fumana*-dominated vegetation.

Keywords: invasion success, open sand grassland, degraded and natural vegetation, soil conditions, transect,

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Introduction

One of the successful invaders of the seminatural open sand grasslands in the „Duna–Tisza köze" region (Danube–Tisza Interfluve) is the perennial grass *Cleistogenes serotina*. Although this species is a native member of the Hungarian flora, it was first recorded from the Great Hungarian Plain only in the middle of the 20th century from two localities, but recently the species has been found in more and more sites (Soó 1973, 1980, Szujkó-Lacza and Kováts 1993, Tölgyesi 1980, Molnár *et al.* 1999). In the recent decade *Cleistogenes* has occupied huge areas of high conservational value in some parts of the Kiskunság National Park (Bagi 1997a, Molnár *et al.* 1999). This vegetation process might considerably alter the endemic open sand grassland vegetation of the region (Bagi 1997a).

In order to make good predictions about the further spread of an invasive plant and about the future changes in the invaded vegetation it is necessary to identify the community types and abiotic conditions that the invader prefers. The preferences of *Cleistogenes serotina* in terms of spread rate in resistant and susceptible plant associations has already been investigated at a relatively broad scale by Bagi (1997a), and he found varying success in different community types. The aim of our study is to detect how the preference and success of the species in terms of relative dominance is related to different gradients in vegetation composition and soil properties at a fine scale and what are the characteristics of the invader-dominated patches as compared to the disturbed and the semi-natural vegetation types of the habitat.

Materials and methods

Research area

Our examinations were carried out in the first two weeks of August in 1996 on the slope of a sand hill in territory IV of Kiskunság National Park near Fülöpháza (20 kilometres west of the city of Kecskemét) in the „Duna–Tisza köze" region in Hungary.

The climate of the area is of moderate continental type. The mean annual temperature is 10–11°C with –1.5 – –2°C in January and 21–22°C in July. The mean annual precipitation is about 550 mm with a maximum in June and November. The number of sunny hours is about 2050 (Iványosi 1979, Borhidi 1993). The water balance is negative, which was extremely expressed in the drought period between 1985 and 1995 (Iványosi 1994).

The historical data show that the main land use form in the latest centuries was grazing which led to the reactivation of sand dunes in the 18th century or before. After the afforestation efforts that culminated in the 20th century and the changes in land use management the sand immobilization began. In the study area intensive grazing went on until the middle of the 20th century, therefore the sand grasslands of the area represent an early phase of the revegetation processes and the immobilization of sand (Magyar 1960, Biró and Molnár 1998).

The sand dunes of the area consist of basic wind-blown sand of Danubian origin (Járai-Komlódi 1966, 1969, Babos 1958). The soil of the sand hills is a very poor basic skeleton soil, without expressed soil profile differentiation. The clay and colloid fraction is extremely low, the humus content is less than 1 per cent. The calcium-carbonate content is considerable, the pH measured in distilled water is around or above eight. The concentration of nutrients is very low. The soils of the depressions have slightly higher humus fraction and lower reaction (Bagi 1997a, Szabolcs 1979, Várallyai 1993).

The vegetation of the study area was mapped in 1990 (Bagi unpublished). Most of the area is covered by different types — *Festuca vaginata*, *Fumana procumbens*, *Stipa capillata* and *borysthenica*, *Salix repens* ssp. *rosmarinifolia* dominated types — of the open perennial sand grassland, with patches of the open annual sand grassland dominated by *Secale sylvestre* and *Bromus tectorum*. According to the latest concept of sand vegetation dynamics (Fekete 1992) the *Festuca vaginata* grassland is the — permanent — natural pioneer plant association on bare sand, while the *Bromus tectorum–Secale sylvestre* type, which was earlier thought to precede the perennial grassland in natural succession (Hargitai 1940, Zsolt 1943, Magyar 1933), develops where degradation — mainly grazing, trampling, ploughing, drying — destroys the original vegetation and nutrients accumulate in the soil (Bagi 1997b, Molnár 1999, Borhidi 1999). In spite of its pioneer character, the open perennial grassland is very rich in rare and specialist species, the number of endemic species (including the dominant grass *Festuca vaginata*) is especially high. The transformation of the annual open sand grassland into a perennial grassland is a slow and uncertain process (Bagi 1990).

The different types of the perennial sand grassland reflect the heterogeneity of the sand dunes: The extremely open *Fumana procumbens* dominated vegetation is found in dry, steep, south facing slopes where sand is still in motion. In the depressions where ground water is close the meadow-like *Salix repens* ssp. *rosmarinifolia* type is found. The habitats of the *Festuca vaginata* and *Stipa capillata* and *Stipa borysthenica* dominated vegetation are less easy to describe, but the latter tends to be more closed and develops on drier soil less disturbed by the wind (Biró and Molnár 1998, Hargitai 1940, Magyar 1933).

In the research area *Cleistogenes serotina* was first found in the end of the 1970's by G. Fekete. During the last decade the species has shown rapid invasion into the *Festuca vaginata*, *Fumana procumbens*, *Stipa capillata* and *S. borysthenica* dominated types (the latter two are more frequent) of the open perennial grassland, while the degraded *Secale sylvestre* and *Bromus tectorum* dominated vegetation has proved to be more resistant. In the invaded vegetation *Cleistogenes* was evaluated to be a facies-forming species up to 28 per cents absolute cover in 10×10 m quadrats. Using the above scale no significant relationship was found between the dominance of *Cleistogenes serotina* and the soil parameters (Bagi 1997a).

Study species

Cleistogenes serotina, a perennial grass is a native member of the Hungarian flora. The geographical range of the species in Europe shows submediterranean characteristics: the northest limits of its area are in Austria, the Czech Republic and Slovakia. *Cleistogenes* can also be found in the east: in the Ukraine, in the region of the Krim and in the Caucasus (Hegi 1935). According to the literature the Hungarian populations belong to the *serotina* subspecies (Soó 1973).

The original habitats of the species in Hungary are dry mountain grasslands on south-facing slopes. In closed limestone or dolomite grasslands

Cleistogenes is often dominant or co-dominant (Soó 1973). The ecological indicator values used in Hungary show that *Cleistogenes serotina* frequents relatively dry, warm, nutrient-poor, calciferous sites (Borhidi 1995, Simon *et al.* 1992, Zólyomi *et al.* 1967). Soó in addition mentions that the species lives on skeleton or silt soils with moderate or poor humus fraction developing on limestone or dolomite, occasionally on andesite (Soó 1973, 1980).

Vegetation sampling

In order to describe spatial gradients in vegetation and soil we used the transect method. The transect was laid on the slope of a south-south-east facing sand hill, running from the top to the bottom with a 10 m long break in the middle which divided the transect in two sections consisting of 70 and 69 0.5×0.5 m quadrats, respectively. The starting point of the transect lies 6.5 metres higher than the end. Thus, the first section represents the higher, the second section represents the lower parts of the slope. The gradient of the slope is uniformly steep in the first section, while the second section gradually flattens out.

Plant cover was collected from 0.5×0.5 m quadrats, the arrangement of which enables to join them into 1 m^2 quadrats overlapping by one quarter with each other. Thus the axis of the transect is the diagonal of the consecutive 1 m^2 quadrats.

The per cent absolute cover of living plants, the dead parts of the vernal aspect, and the criptogams was estimated in each 0.5×0.5 m quadrats. The data might be biassed in the case of the species of the vernal aspect, the cover of which was estimated from the dry stalks except from *Poa bulbosa* (cover of the bulbs). The abundance of *Medicago minima* was estimated on the basis of the number of fruits. The latter species was omitted from the mathematical analyses in which the same type of input data was required for each species.

Soil analysis

Soil samples were taken from the centre of every 1 m^2 quadrats from the top 5 centimetres of the soil. The samples were collected two days after vegetation sampling, one day after rain. Each soil sample was put into plastic bags, and weighed one day later. After that the samples were dried out at 60 °C and weighed again. Soil humidity is expressed as the percentage weight difference of the collected and the dried soil. Organic matter was determined with dichromatic method with photometric evaluation. $CaCO_3$ content was measured by calcimeter of SCHLEIBER. Soil reaction was determined from a 1:2.5 water suspension with an electric pH meter. Available phosphate and potassium were extracted from the soil by ammonium-lactate. Phosphate was determined with ammonium-molibdenate by colorimetry, potassium content was determined by flame photometry. Avaliable nitrate was measured from a 1:5 water suspension with phenol-disulphonic-acid by colorimetry (Ballenegger 1953). All measurements were performed in one replicate.

Mathematical evaluation

The ordination of the species was performed by nonmetric multidimensional scaling (NMDS) on the basis of the cover in the 0.5×0.5 m quadrats, while the vegetation was examined by principal components analysis (PCA) of the cover in the overlapping 1×1 m quadrats (the 0.5×0.5 m quadrats yield the same result, but in that case the interpretation is more complicated). The calculation of the ordination was based on a correlation matrix in both cases, the number of dimensions was three.

Correlations between soil parameters and relative elevation were estimated using Spearman's rank order correlation coefficient R_s. The difference of the soil parameters among groups of quadrats separated by the PCA analysis were tested using the Kruskal-Wallis test, pairwise comparisions between groups were calculated by Dunn's method (Zar 1984). For the calculation of the statistical analyses and ordinations we used Statistica for Windows program package (Statsoft Inc. 1995).

Results

The vegetation of the whole transect consists of 24 phanerogamous plant species. At the time of the sampling 15 of them had living aboveground parts. The vernal aspect was represented by 10 species. Only *Medicago minima* was present in both groups (abundant in the vernal aspect, but having merely one living specimen). Half of the species was present in less than 5 per cents of the primary 0.5×0.5 m sampling units. The cryptogams were represented by one moss species (*Tortula ruralis*) and three lichens (Table 1).

The abundant species quite well define the vegetation types along the transect (Fig. 1): *Fumana procumbens* is the dominant species in the first part of the first and second section of the transect (1–12, 23–28). *Stipa borysthenica* achieves its greatest cover values in the first part of the second section of the transect (24–34), while *Secale sylvestre* becomes gradually the most abundant in the second part (30–

Table 1. Species list for the two sections of the transect. The number of occurences are given for the 0.5×0.5 m quadrats as well as the median of the per cent cover data for the quadrats where the species was present. Where the species was present only in one quadrat, the cover value is given in parentheses.

SPECIES	CODE	NUMBER OF OCCURENCES			COVER MEDIAN	
		Sect. 1	Sect. 2	Total	Sect. 1	Sect. 2
Living plants						
Cleistogenes serotina (L.) Keng	CLES	67	43	110	10	8
Stipa borysthenica Klokov	STIB	51	50	101	3	6
Fumana procumbens (Dun.) Gren. et Godr.	FUMP	43	16	59	10	9
Festuca vaginata W. et Kit.	FESV	4	1	5	4.5	(15)
Alyssum tortuosum W. et Kit.	ALYT	7	0	7	1	0
Salsola kali L.	SALK	2	10	12	11.5	10
Polygonum arenarium W. et Kit.	POLA	10	1	11	3.5	(2)
Bothriochloa ischaemum (L.) Keng	BOTI	1	0	1	(1)	0
Alkanna tinctoria (L.) Tausch	ALKT	2	4	6	0.75	5
Stipa capillata L.	STIC	3	1	4	8	(3)
Koeleria glauca (Schkuhr) DC	KOEG	4	2	6	3	6.25
Euphorbia segueriana Necker	EUPS	3	0	3	1	0
Corispermum nitidum Kit.	CORN	0	1	1	0	(2)
Conyza canadensis (L.) Cronq	CONC	1	14	15	(1)	2
Vernal aspect						
Medicago minima (L.) Grufbg.	MEDM	9	50	59	–	–
Poa bulbosa L.	POAB	54	13	67	15	5
Buglossoides arvensis (L.) I. M. Johnst.	LITA	39	69	108	2	5
Arenaria serpyllifolia L.	ARES	29	56	85	1	2
Minuartia verna (L.) Hiern.	MINV	4	0	4	1.5	0
Silene conica L.	SILC	4	26	30	1	4
Cerastium semidecandrum L.	CERS	2	1	3	1.5	(1)
Alyssum turcestanicum Regel et Schmalh	ALYD	1	1	2	(0.5)	(0.5)
Secale sylvestre Host	SECS	0	65	65	0	10
Bromus squarrosus L.	BROS	0	43	43	0	3
Cryptogams						
Tortula ruralis (Hedw.) Gaertn. *et al.*	TORR	51	79	130	1	15
Cladonia convoluta (Lam.) P. Cout.	CLAC	69	70	139	1	1
Parmelia pokornyi (Koerb.) Szat.	PARR	49	69	118	3	1
Collema crispum (Huds.) Web.	COLC	30	14	44	0.5	0.5

45), with other degradation-indicating species like *Bromus tectorum*, the moss *Tortula ruralis* and the adventive *Conyza canadensis*.

The invader *Cleistogenes serotina* is present in almost all quadrats except those at the end of the transect, where degradative species predominate (Fig. 1). The species shows two marked peaks: the greatest cover is found at the second part of the first section (10–19), while a much smaller peak is found in the second section (35–39).

Small subordinate annuals like *Buglossoides arvensis, Arenaria serpyllifolia, Medicago minima* though achieve their maximal abundance in the company of *Secale sylvestre* have a smaller peak in the first section (10–21) where *Cleistogenes* predominates. The ephemerous perennial *Poa bulbosa* displays a peculiar trend: its greatest abundances in the first section almost overlap with the peak of *Cleistogenes serotina*. This species is not present in the second section except from some quadrats where the cover of *Secale sylvestre* shows local minimum (Fig. 1).

The PCA ordination of the 46 quadrats (Fig. 2) reveals three consistent groups, which can be corresponded to the *Fumana*- (Fig. 2: A-group, 1–13, 24–26), *Secale*- (Fig. 2: C-group, 31–46) and the *Cleistogenes*-dominated (Fig. 2: B-group, 14–23) types. Both sections of the transect start from the *Fumana*-dominated type. The transition towards the *Cleistogenes*-dominated group is relatively sharp in the ordination space while there is a continous transition towards the *Secale*-dominated type (Fig. 2).

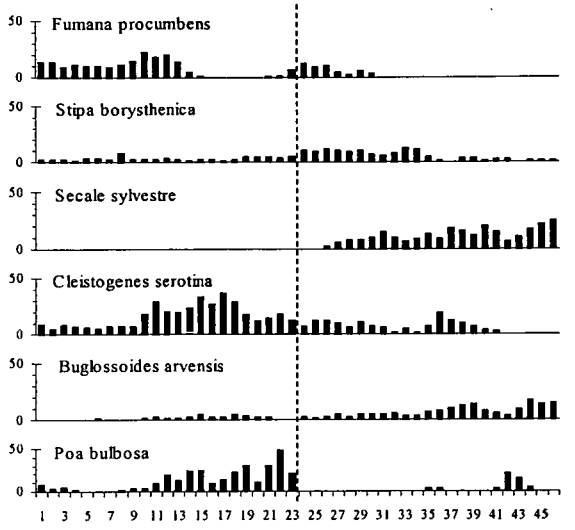

Fig. 1. The per cent cover of some representative species along the transect in the 1×1 m quadrats. The two sections are separated by the broken line.

Nonmetric multidimensional scaling of the species evaluating the 0.5×0.5 m quadrats is performed for all quadrats (Fig 3a) and also separately for the two sections (Fig. 3b and 3c) of the transect, as the representatives of two different vegetation transitions according to the PCA results. Most of the vernal annuals form a consistent group together with the moss *Tortula ruralis* and the invasive annual *Conyza canadensis*, while *Fumana procumbens* and *Cleistogenes serotina* are at the opposite end of the first axis when the whole transect (Fig. 3a) or the second section (Fig. 3c) is analyzed.

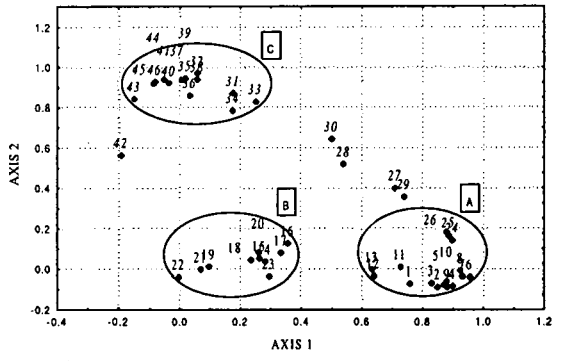

Fig. 2. PCA ordination of the 1×1 m quadrats along the transect on the basis of the cover of the phanerogamous plants. The per cent variance represented by the first two axes are 40.62 and 28.90, respectively. The serial number of the quadrats belonging to the second section are written in italics. A: *Fumana*-type, B: *Cleistogenes*-type, C: *Secale*-type.

Evaluating the first section *Cleistogenes serotina* and *Fumana procumbens* form two opposite poles along the first dimension. The position of cryptogams and the small therophytes like the vernal *Arenaria serpyllifolia, Buglossoides arvensis, Medicago minima*, and the summer annual *Polygonum arenarium* show in the ordination space that these species are more abundant in the *Cleistogenes*-dominated quadrats (Fig. 3b).

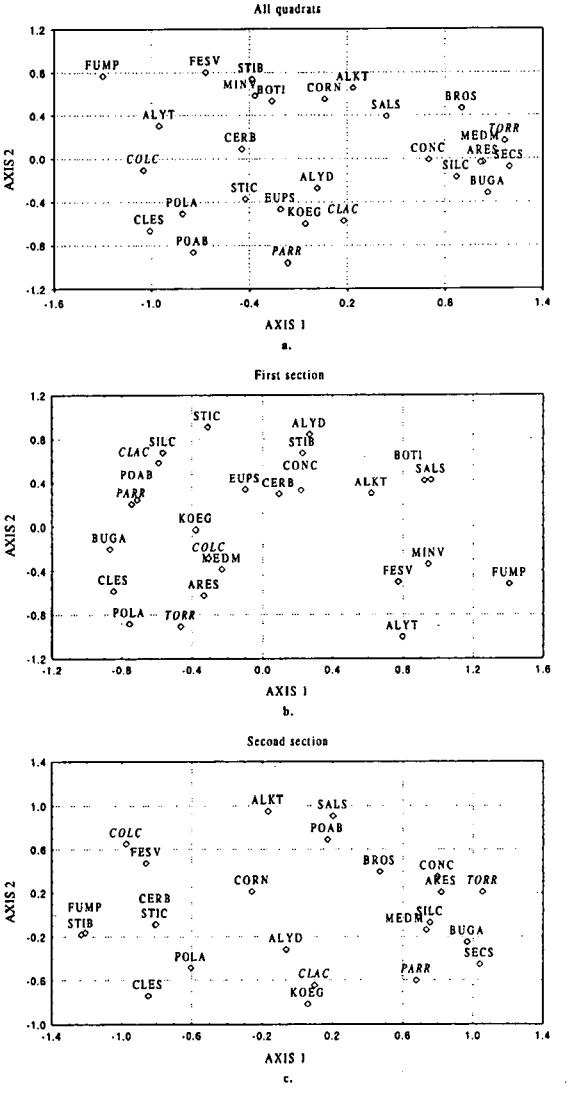

Fig. 3. The NMDS ordination of the species on the basis of the cover in the 0.5×0.5 m quadrats in the whole transect (a.), and separately for the first (b.) and the second section (c.) of the transect. STRESS = 0.1446 and 0.1674 and 0.1405 respectively. For the abbrevations see Table 1. The codes of the cryptogams are written in italics.

The values of the measured soil parameters along the transect are seen in Fig. 4. Three of the soil parameters are significantly correlated with relative elevation (carbonate content: $R_s = 0.589$, $p < 0.001$;

Table 2. The sum and the average of ranks for seven soil parameters in the three groups of quadrats separated by PCA analysis (see Fig. 2) and the results of the Kruskal-Wallis test. The groups of quadrats are marked A, B and C as in Fig. 2. The soil parameters are: WAT: soil water content, HUM: soil organic matter content, PH: soil pH, CARB: soil carbonate content, P: soil phosphate content, K: soil potassium content, N: soil nitrate content. The significance values for the H statistic are: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$.

	A (n = 16)		B (n = 10)		C (n = 15)		H
	Sum Rank	Aver Rank	Sum Rank	Aver Rank	Sum Rank	Aver Rank	
WAT	252	15.750	326	32.600	283	18.867	12.927**
HUM	277	17.313	225.5	22.550	358.5	23.900	2.566 ns
PH	404.5	25.250	254	25.400	202.5	13.500	9.303**
CARB	479	29.938	183.5	18.350	198.5	13.233	15.716***
P	193.5	12.094	190	19.000	477.5	31.833	21.400***
K	331.5	20.719	254.5	25.450	275	18.333	2.142 ns
N	215	13.438	165.5	16.550	480.5	32.033	20.499***

Table 3. The difference between the rank averages of the three groups separated by PCA analysis (see Fig. 2) and the results of the Dunn's test for the soil parameters that yield significant result in the Kruskal-Wallis test (cf. Table 3.) . The groups of quadrats are marked A, B and C as in Fig. 2. For the abbrevations see Table 3. The significance values for the Q statistic are: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$.

	A vs. B		A vs. C		B vs. C	
	Diff Ranks	Q	Diff Ranks	Q	Diff Ranks	Q
WAT	16.850	3.489**	3.117	0.725 ns	13.733	2.808*
PH	0.150	0.031 ns	11.750	2.735*	11.900	2.438*
CARB	15.588	2.402*	16.704	3.883***	5.117	1.047 ns
P	6.906	1.430 ns	19.740	4.586***	12.833	2.625*
N	3.113	0.645 ns	18.596	4.323***	15.483	3.168**

phosphate content: $R_s = -0.666$, $p < 0.001$; nitrate content: $R_s = 0.645$, $p < 0.001$). The correlations suggest that soil phosphate and nitrate tend to accumulate at the bottom, while carbonate concentration is higher at the top of the slope.

The consistent groups of quadrats separated by PCA were analyzed for differences in their soil properties. The Kruskal-Wallis test shows that the three vegetation types do not differ significantly in their soil organic matter and potassium content (Table 2). The pairwise comparisions reveal that the most marked differences are between the soils of the *Fumana*-dominated and the the *Secale*-dominated groups. The soils of the *Cleistogenes*- and the *Fumana*-dominated types have significantly different water and carbonate content, while the difference between the soils of the *Cleistogenes*- and the *Secale*-dominated group is the nitrate, phosphate, and water content and soil reaction (Table 3).

Discussion

The analysis of the transect detected three quite distinct vegetation types. Two of them, the *Fumana procumbens-Stipa borysthenica*-dominated quadrats at the top and the middle of the slope represent the original seminatural perennial grassland type while the *Secale sylvestre*-dominated quadrats with high moss cover, at the bottom of the slope, represent the degraded annual grassland type of the habitat. The soil of the latter type has higher nutrient concentration, lower pH and carbonate content. The invader *Cleistogenes serotina* is present in both types with relatively low cover, though it seems to be less successful where the representatives of the degraded annual type predominate at the end of the transect. Interestingly, at the bottom of the slope, where *Cleistogenes* disappears there are practically no summer green plants except *Stipa borysthenica* at very low densities and another invasive species *Conyza canadensis*.

The third type, the *Cleistogenes serotina*-dominated quadrats are also found at the higher part of the slope. In this type *Fumana* disappears, and the cover of the small subordinate annuals and cryptogams is slightly higher than in the *Fumana*-dominated quadrats but is lower than in the *Secale*-type. The soil conditions are more similar to the *Fumana*-dominated type (low nitrate and phosphate concentration and high soil pH) although carbonate concentration is lower. As the main difference we measured the highest water content of the top soil in the *Cleistogenes*-type.

Our results agree with the conclusions of Bagi (1997a) who detected the highest spread rate in the *Fumana*- and *Stipa*-dominated vegetation, and slow

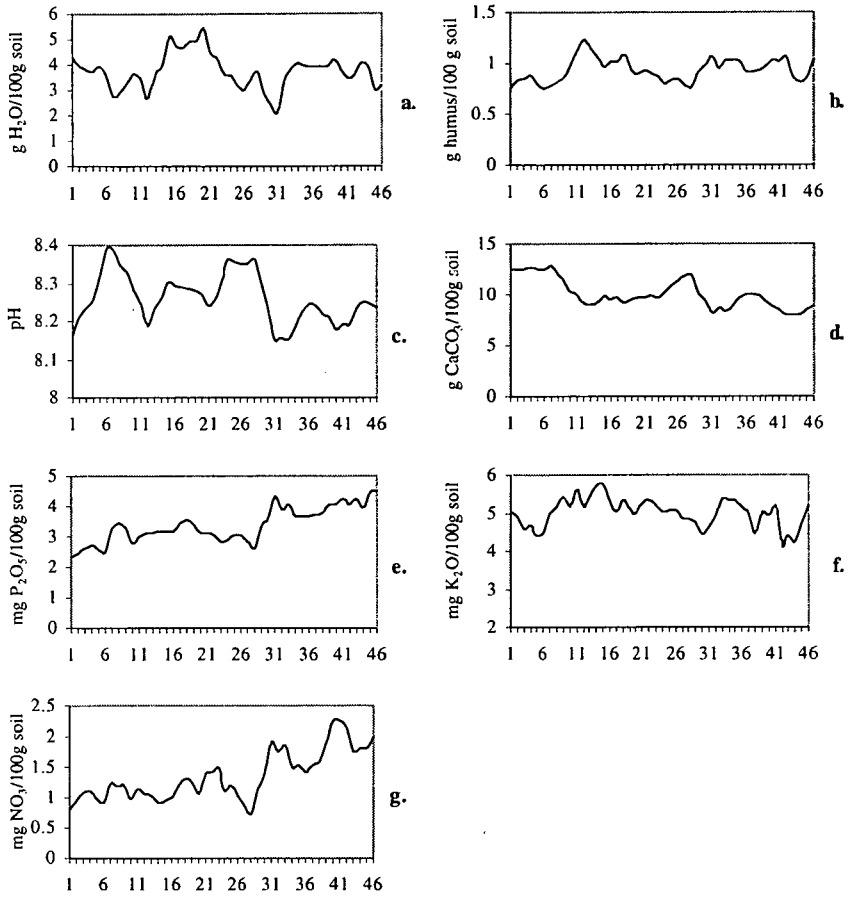

Fig. 4. The distribution of the soil parameters (represented as sliding average) along the transect: (a): actual water content, (b): organic matter content, (c): pH in distilled water, (d): carbonate content, (e): available phosphate content, (f): available potassium content, (g): nitrate content.

spread in the *Secale-Bromus*-dominated type. The soil conditions of *Cleistogenes*-dominated type detected by our transect suggest that the invader is most successful in terms of dominance in patches that are similar to the habitat of the *Fumana*-type except from the slightly more favourable water conditions. Other studies have proved that water is one of the the most important limiting factors in these communities (Kovács-Láng and Szabó 1973, Szabó 1975), but it should be mentioned that our results are founded on one measurement and refer only to the top five centimetres of the soil, which is not comparable to the actual root depth of many species (Magyar 1933, Simon and Batanouny 1970), therefore care should be taken when our data are concerned.

The studies on invasions traditionally emphasize that exogenously disturbed habitats are more prone to invasions (Fox and Fox 1986, Usher 1988, Drake *et al.* 1989, Hobbs and Huenneke 1992, Beerling 1995). Although this phenomenon in many cases seems trivial, the interpretation that relates invader success to disturbance status or degradation of the target community in our case is not relevant. The invasion process of *Cleistogenes serotina* has apparently not been mediated by exogenous disturbance at the relatively intact parts of the strictly protected study area since it was first detected. Nonetheless, the perturbation of the soil probably promoted colonization inside some sites, mainly along the roads (Bagi 1997a). Interestingly the two invasive species that are present in our transect show different behaviour: the focal species of our study, *Cleistogenes serotina* seems to be more successful in the natural, nutrient-poor open perennial grassland type while *Conyza canadensis* is found in the degraded open annual grassland type where relatively more nutrients accumulate.

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TISCIA 32, 19-29

VARIABILITY OF SPATIAL DEPENDENCE WITHIN A PIONEER PLANT COMMUNITY

A. Horváth and L. Makrai

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Abstract. Since it is important to know the degree of variability of spatial dependence among plant populations when comparing two communities, but only a very few literature data exist on it, the first aim of this study is to give some compensation for this deficiency. We applied information statistical models to estimate vegetation pattern organization in an early stage of primary succession, and computed associatum values and their variances along the spatial scale. The null model was the random shift, the validation procedure was also executed for it. We can summarize, that the sampling area with 50 m^2 was sufficiently large to gain interpretable associatum curves. The area of maximum spatial organization was 15 cm × 15 cm, which significantly differs from null model. When we divided the whole sampling area to 10 m^2 stripes, the associatum difference curves of every parcels had significant interval, and average of them was similar to the curve from the whole area. Therefore, a 10 m^2 area could represent the unit of entire community. When the area was decreased to 2.5 m^2, the variability of spatial dependence increased, and the appearance of significant associatum values became more unpredictable. At this size of area the unity of patterns organization seems to be collapsed, and the samples can not represent effectively the entire community. Six types of associatum measures were compared regarding to their variability in the spatial series steps.

Keywords: information theory models, syncretic functions, spatial dependence, spatial scaling, primary succession, randomization tests, validation of null models

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Introduction

Since the beginning of nineties, after the methodological foundation by Juhász-Nagy (1972, 1976, 1980, 1984), the application of the information statistical models started to get into the practice of the spatial pattern analysis. In addition to solving methodical problems (e.g. computation: Bartha *et al.* 1994, Erdei and Tóthmérész 1993, Podani 1993, Horváth 1998), some case studies were also carried out. These experiments produced some evidences about the pattern transformation during primary succession (cf. Bartha 1990, 1992, Margóczi 1995, Horváth 1997), or, e.g., spatial dependence of populations in different types of loess steppe (Hochstrasser 1995), dolomite grassland communities (Szollát and Bartha 1991, Bartha *et al.* 1998) or prairie vegetation (Bartha *et al.* 1995).

In these studies two or more plant communities were compared on the basis of main syncretic functions, characteristic scaling, or plexus graphs of pairwise associations. Generally, each community types (e.g. successional stage) were represented by only one sample. Though the field sampling procedures were very extensive in all cases, and the samples consisted of several hundreds or thousands of primary plots (microquadrats), the statistical evaluation of comparison between communities was impossible because of missing repetition of samples. For correct separation of different stands it is unavoidable to know the variation of adequate coenological characteristics.

There is only a few field experiment that yielded some facts about the variability of syncretic functions of a given community. Hochstrasser (1995) studied seven types of loess grasslands, four

of which were examined in *two different (consecutive) years*. She observed relatively large change between two years in maximum associatum and relative associatum (both from field and random difference values), which may be originated only partly from secondary successional dynamics. The temporal shift of associatum values makes the unambiguous distinction of community types impossible. So, in her study, the temporal dynamics (trend, periodicity or stochasticity) of spatial dependence could be greater than its difference between coenotaxa, whereas their floristic and physiognomic diversity was significant.

Bartha *et al.* (1998) compared two dry dolomite grassland types existing on contrasting slopes. One of them was represented by four, the other one by five samples collected from different dolomite hills. Mann-Whitney method was applied for testing the null hypothesis that the aspect had any effect on some basic syncretic functions. The results showed that significant differences between two grassland types could be detected when they were restricted to assemblages of only dominant species. Otherwise, analysing the entire communities, the variance of structural characteristics was quite large. In this study, however, the sample repetitions for a community types were collected from *different stands*, from different floristic situations, that could be one component of the great variability.

We can find some data about variance of structural dependence relating to *one stand* of one community only in the work of Bartha *et al.* (1995). The subject of this study was to compare three prairie communities with two samples for each. It has been pointed out that there was no clear difference between two stands regarding the relative associatum curves, only the third grassland type was separable from the others. The correlation between total transect lengths (each sample was divided to two part, and they were fused, respectively) and maximum relative associatum was not significant.

Some general, partly methodical questions or problems emerge from the results cited above:

(1) What is the size of a *sufficiently large sample area* (grid or transect) that represents the entire (and more or less homomorfous) stand of a given community for comparison to other ones? The question is related to the problem of variability of vegetation pattern along spatial scale. In this effect, the scaling problem is independent of *characteristic scaling*, because now the aim is just to find the smallest part of a vegetation stand that gives constant characteristic values (if they are), independently its location in the stand.

(2) How large is the variance of spatial dependence in a community when its pattern is divided into some parts? How does this variance depend on the size of the partial patterns?

(3) Can the average of associatum functions of different parts approach the spatial dependence in entire community?

(4) Is there any significant difference between average (or median) of values coming from parcels of field sampling area and randomized patterns?

We will focus on the last three problems. We suppose that our relatively extensive field sample represents the studied community, therefore the main aim will be to get some evidences about the variability of mutual spatial dependences. We compare the variance of field values with random patterns even when randomizations are related not exactly to same parcel, but the other part of the given stand. We use the random shift null model to make random patterns, and, for correct statistical evaluation, we give the validation of the test.

Materials and methods

Study site

The study site was located on the plateau of a spoil bank consisting of sterile clayey bedrock, which originated from an opencast coal-mine at Hepworth (near Huddersfield), West Yorkshire, England. The field sampling was carried out in first half of May, 1993. Since the stand was only several years old, the development of vegetation could reach the stage of a pioneer community, so it could be considered as an early stage of primary successional process. The apparently primitive grassland had no complex physiognomical structure, it consisted of only two simple layers. The lower one was composed of mosses and lichens, while the upper one could be specified as the composition of grasses and some dicotyledons.

The population number was very low, there were only ten taxa in the studied area. Only two species (*Pogonatum urnigerum* and *Agrostis capillaris*) reached more than 10 % of frequency regarding the whole sampling area, otherwise they were present at least every third cell of the grid (Table 1). Only the half of the species were vascular plants, three of which were grasses. Dominant grass was *Agrostis capillaris*, subdominant was *Deschampsia flexuosa*, which have circumpolar area type, and they are common in England. Both species exhibited clumped pattern (Fig. 1); the tussocks of *Deschampsia* are smaller and more segregated.

Table 1. Taxon list of the studied community. The frequency values are calculated from whole sampling area of 20000 plots.

Code	*Taxon name*	*Frequency (%)*
Pu	*Pogonatum urnigerum* (moss)	39.68
Ac	*Agrostis capillaris*	35.26
Gp	*Grimmia pulvinata* (moss)	7.24
Df	*Deschampsia flexuosa*	5.50
Dc	*Distichium capillaceum* (moss)	5.25
Ra	*Rumex acetosella*	4.06
Cp	*Ceratodon purpurascens* (moss)	3.26
Ep	*Epilobium* sp.	1.59
Li	*Lichenes*	0.86
Hl	*Holcus lanatus*	0.72

Primary and secondary sampling

The sampling area was a 10 m × 5 m grid (50 m^2) with 5 cm × 5 cm microquadrats (primary plots), total number of *primary plots* was 20000 (200 × 100). We recorded the presence or absence data of each species in all cells. For studying the variability of pattern characteristics at relatively large area, we established five uniform, non-overlapping *stripes* on the grid, which are marked with upper case letters (A-E). Then we divided each stripe into four *blocks* (signed lower case letters, a-d, cf. Fig. 1), which can still provide sufficiently large sample size. The area of each stripe is 10 m^2 (10 m × 1 m), while the blocks are 2.5 m^2 (2.5 m × 1 m).

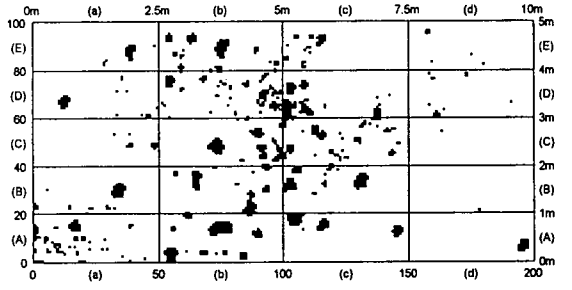

Fig. 1. The pattern of *Deschampsia flexuosa*. The numbers of cells on the grid are indicated at bottom and left, while the distance in meters at top and right. The letters in parentheses mark the stripes of sampling area (A-E) or the blocks of stripes (a-d).

For spatial scaling we performed *systematic secondary sampling* in several *spatial series steps* (Table 2). Given number of contiguous cells of the grid was fused, which formed (except second and fourth step) isodiametric secondary sampling units (cf. Table 2). The number of secondary plots (sample size) was the greatest possible at each steps, so we realized a *complete sampling.*

Table 2. The properties of spatial series steps regarding to analysis of whole sampling area, stripes and blocks.

Step #	*Number of fused primary plots*	*Area of secondary plots (m^2)*	*Number of secondary plots*		
			in whole area	*in stripes*	*in blocks*
1	1 × 1	0.0025	20000	4000	1000
2	2 × 1	0.0050	19900	3980	980
3	2 × 2	0.0100	19701	3781	931
4	3 × 2	0.0150	19602	3762	912
5	3 × 3	0.0225	19404	3564	864
6	4 × 4	0.0400	19109	3349	799
7	5 × 5	0.0625	18816	3136	736
8	6 × 6	0.0900	18525	2925	675
9	7 × 7	0.1225	18236	2716	616
10	8 × 6	0.1600	17949	2509	559
11	9 × 9	0.2025	17664	2304	504
12	10 × 10	0.2500	17381	2101	451
13	12 × 12	0.3600	16821	1701	351
14	14 × 14	0.4900	16269	1309	259
15	16 × 16	0.6400	15725	925	175
16	18 × 18	0.8100	15189	549	99
17	20 × 20	1.0000	14661	181	31
18	24 × 24	1.4400	13629	–	–
19	28 × 28	1.9600	12629	–	–
20	34 × 34	2.8900	11189	–	–
21	40 × 40	4.0000	9821	–	–
22	50 × 50	6.2500	7701	–	–
23	60 × 60	9.0000	5781	–	–
24	70 × 70	12.2500	4061	–	–
25	80 × 80	16.0000	2541	–	–

Random references

To compare the results with null hypothesis – to generate random patterns – we applied the *random shift* methods (Palmer and van der Maarel 1995), and in one case the *complete randomization* (i.e. *independent assignment* in Roxburgh and Matsuki 1999). Using random shift we can analyse the degree of spatial dependence between populations in such a manner that we randomize only the interspecific patterns (the autocorrelation of each population pattern is kept more or less constant), and the results are free from textural constraints (cf. Bartha and Kertész 1998). It is reasonable that in the case of non-stationary patterns (e.g. if the pattern consists of several large patches), the random shift modifies the value of autocorrelation (Palmer and van der Maarel 1995, Roxburgh and Chesson 1998), regarding to our relatively large sample area, however, this problem does not arise (cf. Fig. 1).

Executing Monte-Carlo simulation the number of randomizations was in general 99, but with smaller data sets it was 999. In the statistical evaluation we performed one-sided test using significance level as $p = (n - ND + 1) / (n + 1)$, where n is the number of randomization, ND is the number of positive or negative (the higher) differences between field and random values (cf. Manly 1997).

When we calculate significance level, we have no evidences about the real values of probability of type I error. It can be determined however, if we compare random patterns to other ones originated from same methods, and we calculate probability that a characteristic value of a random pattern significantly differs from others (Roxburgh and Matsuki 1999). In ideal cases, the significance level and probability of making a type I error is same value, but we have to evaluate *validation procedure* to settle whether it is true or not in a given situation (using a given randomization test, and calculating a given characteristic value). In the first part of Results we will give the detailed description of the validation test method and its results for using random shift null model on a grid, and analysing spatial dependence of species patterns.

Measure of spatial dependence and its variability

Using information statistical models, we were interested in the degree of mutual dependence of all populations within the entire community (Juhász-Nagy 1976, 1984). To estimate it, we calculated *associatum* for field patterns (*fAss*) and the *associatum difference* values (*dAss*) along the spatial scale. We can get the *dAss* values if we subtract *aAss* from *fAss*,

$$dAss = fAss - aAss,$$

where *aAss* is the *average of associatum* values regarding random references.

In comparison of different communities with each other, it is worthy to consider the application of *relative associatum* that is defined by the ratio of *associatum* and *florula diversity* from field data:

$$relAss = fAss / fFD$$

(Bartha *et al.* 1995, 1998). This measure can be derived from the so called *simple relative associatum* introduced by Juhász-Nagy (1984: p. 371), as a redundancy estimate given by $1 - fAss / fFD$. In the cited work (p. 385), Juhász-Nagy obtained another formula to calculate redundancy-like relative associatum, better than the previous one. It is signed as *redAss* here, and given by:

$$redAss = 1 - fAss / locEv,$$

where *locEv* is the *local evenness* (see Juhász-Nagy, 1984 for more details).

We will introduce two other relative associatum measures, in relation to null model. Let the first one called *calibrated associatum* (*calAss*). It is „calibrated" between its actually possible maximum and minimum values, which are the *local distinctiveness* from field (*fLD*) and the *average associatum* from random references (*aAss*). The formula is defined as:

$$calAss = (fAss - aAss) / (fLD - aAss),$$

or more simple $calAss = dAss / (fLD - aAss)$. The other measure is the *„sensitive associatum"* (*senAss*), which is more responsive to spatial dependence, because it is defined by the ratio of *associatum difference* (*dAss*) and *dissociatum* from field data (*fDiss*):

$$senAss = dAss / fDiss.$$

The sensitivity can be seen easily, if we consider that *associatum* and *dissociatum* are complementary measures.

For analysing spatial patterns (calculating information statistic functions, performing spatial scaling and making random references) we applied the *INFOTHEM* program (Horváth 1998). All the measures are *standardized by the number of secondary plots* (listed in Table 2).

For estimating the variability we used the *variation coefficient*, which was calculated as a ratio of the standard deviation and the mean of the data. In comparison of associatum values of field and random patterns, since the normality of our data can be rejected only in several cases (*Kolmogorov–Smirnov* and *Shapiro–Wilks' W test*), generally the *two sample t-test* can be applied. The results of both *t-test* and *Mann–Whitney U test* were similar, so we will present only the significance values of the latter one, listed along the spatial scale.

Results

Validating random shift null model

Though Palmer and van der Maarel (1995) referred to the validation process of random models applied to analyse spatial dependence, and Roxburgh and Chesson (1998) also showed the results of this method, the most detailed and correct description can be found in the paper of Roxburgh and Matsuki (1999). All three works applied validation test for *pairwise association*, so it was necessary to extend it to *associatum difference*, as well.

Regarding *random shift* procedure on a grid, we can find some facts about validation in only two works. Palmer and van der Maarel (1995) pointed out that at $p = 0.05$ the probability of type I error is exactly 5 %, so the null model can be validated. Afterwards, Roxburgh and Matsuki (1999)

demonstrated, that when spatial autocorrelation increases, the chance of making type I error also increases. It means that random shift model can be validated, if the degree of autocorrelation is low (Morans $I < 0.4$). We can not fully accept this result, because they confused two different aspects of randomization test. One of them is the question of keeping autocorrelation value constant. From this point of view, the random shift is indeed not a perfect model in case of non-stationary patterns (as it was noted above). However, the question of reliability is the other aspect. It depends on the realization of randomization process and the calculation of the significance level. Thus, if the test produces real probability of type I error, it can be validated even when its applicability is questionable (because of e.g. too few and large patches). For this purpose it is clear that to make *pseudo-observed* patterns (comparing it to random references), they should have to use also the random shift model, which was applied for randomization test, but they did it in another way (Roxburgh and Matsuki 1999).

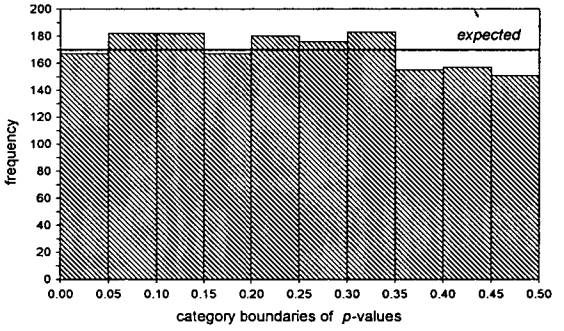

Fig. 2. Frequency histogram of the distribution of *p*-values referring to *dAss*, resulting from validation test of random shift model. Since the number of *p*-values is 1700, the expected frequency at each category is 170. Note, that x-axis ranges to 0.5, because of the applied formula of significance level.

We accomplished the validation test for random shift model with keeping the above notes in mind. We chose the stripe A from the whole grid, and executed random shift procedure on its field pattern making random patterns. The number of such *pseudo-observed patterns* were 100 (each contained 4000 cells). For each pattern the *dAss* curves were calculated using 99 randomizations (also with random shift), and then the significance level was computed. Since there is no real spatial dependence in pseudo-observed patterns, a significant *dAss* value can be detected as a result of random coincidence. Therefore we expected the probability for a significance level to fall constantly within the different *p*-value categories. The result must be a *rectangular distribution* of the frequency histogram, as we can see on Fig. 2, which is made by accounting all 17 spatial series steps from 100 pseudo-observed patterns). To detect any departure from rectangular distribution, a *Kolmogorov-Smirnov one-sample test* and χ^2*-test* were applied, which gave non significant values ($d_{max} = 0.0276$, $p < 0.2$; $\chi^2 = 8.0353$, $p = 0.43$). When we calculated d_{max} and χ^2 for each spatial series steps separately, we got also non significant deviation in all steps.

For completing the validation test the calculation of the probability of type I error is also necessary. We chose two significance level ($p = 0.01$ and $p = 0.05$), and counted the number of significant positive or negative *dAss* values at each spatial series step. The frequencies, given as relative values, are exactly the rate of type I error, because no significant differences are expected between pseudo-observed (actually random) patterns and their random references. It can be seen in Table 3, that the probability of type I error corresponds to both significance levels (considering both negative and positive differences). A *Wilcoxon matched pairs test* was applied to detect statistically any difference between observed and expected relative frequencies of significant *dAss* values along spatial scale. The test confirmed that the type I error rate approximated the chosen *p* level (Table 3).

Table 3. The relative frequencies of significant negative (*rFSD* –) or positive (*rFSD* +) *dAss* values from 100 pseudo-observed patterns, at two significance levels. The Wilcoxon *T* for differences between observed (as probability of type I error) and expected *rFSD* values (given by *p*) are also listed.

Area (m^2)	$p = 0.01$		$p = 0.05$	
	rFSD –	*rFSD* +	*rFSD* –	*rFSD* +
0.0025	0.01	0.01	0.05	0.02
0.0050	0.03	0.01	0.06	0.02
0.0100	0.00	0.00	0.05	0.05
0.0150	0.01	0.00	0.07	0.04
0.0220	0.01	0.01	0.07	0.05
0.0400	0.01	0.01	0.06	0.04
0.0630	0.00	0.01	0.04	0.03
0.0900	0.01	0.02	0.04	0.03
0.1200	0.00	0.02	0.04	0.05
0.1600	0.01	0.00	0.05	0.04
0.2000	0.01	0.00	0.07	0.03
0.2500	0.00	0.00	0.03	0.05
0.3600	0.02	0.02	0.04	0.09
0.4900	0.01	0.03	0.04	0.05
0.6400	0.03	0.03	0.06	0.06
0.8100	0.00	0.01	0.08	0.09
1.0000	0.01	0.01	0.02	0.06
average	0.0100	0.0112	0.0512	0.0471
Wicoxon T	17.5	27.5	57.0	30.0
p for Wilcoxon T	0.944	0.625	0.865	0.279

This and the previous results show, that applying the random shift model in a statistical test, it is neither too liberal, nor too conservative. So, regarding to *dAss* values, the model can be validated. Note, that the validation process was successful even when only 99 randomizations were executed.

Characteristics of whole sampling area

We have calculated the basic syncretic functions along the spatial scale to characterize entire community regarding to whole sampling area of 10 m × 5 m grid (Fig. 3a). We have also computed the difference values using random references (Fig. 3b-c). It can be seen on Fig. 3a, that the *fAss* values are very small compared to other functions, but not zero. Fig. 3a shows, that only low degree of spatial dependence appears agreeing with pioneer community, but it can be detected even if only ten taxa are present. Characteristic area of *fLD* (as compensatory area, A_{comp}), *fFD* (as A_{flor}) and *fDiss* is 0.16 m^2 equally, however for *fAss*, A_{ass} = 0.49 m^2. The minimum area (in the sense of Juhász-Nagy and Podani 1983), A_{min} = 12.25 m^2. The ordering of maximum areas gives the relation as

$$A_{flor} = A_{comp} < A_{ass} .$$

The *characteristic interval* is located between 0.01 m^2 and 0.64 m^2.

Analysing the *dAss* curve coming from complete randomization procedure, it can be almost the same as *fAss* values (Table 4, Fig. 3b). This phenomenon is due to the given null model which make the spatial dependence among species completely impossible, and indicates that the textural constrains are negligible in our sample. Whereas, the influence of patchiness of species pattern can not be neglected, because the *dAss* curve arising from random shift model is sufficiently different (cf. Table 4 and Fig. 3c). Significant *dAss* values appear at smaller areas, from 0.0025 m^2 to 0.09 m^2, and the maximum values of them is located at 0.0225 m^2. It means, that the organization of community pattern reaches its maximum value in a 15 cm × 15 cm area.

Table 4. The characteristic associatum values and their areas for whole sample area.

Function	*maximum value (bit)*	*maximum area (m^2)*
FAss	0.435	0.4900
dAss from complete randomization	0.430	0.4900
dAss from random shift	0.142	0.0225

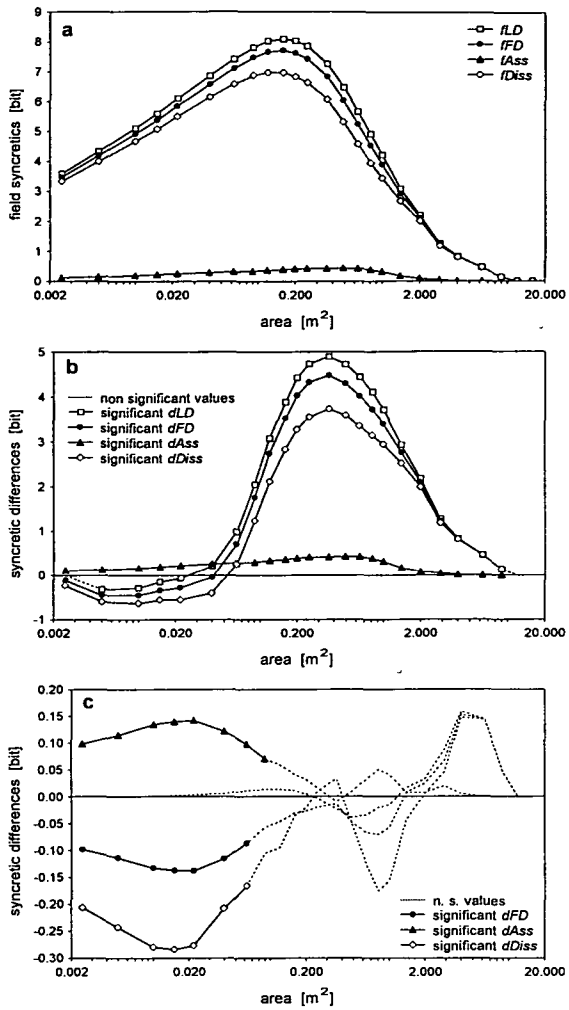

Fig. 3. Syncretic functions (a) and their difference values (b-c) from whole sample area. Random reference was represented by 99 compete randomizations (b), and random shifts (c) respectively. The significance level, $p = 0.01$. There are no significant *dLD* values in case of random shift, because this null model does not change the autocorrelation of species patterns.

Variability of associatum of stripes in relation to whole sampling area

Before analysing spatial dependencies among populations in the five stripes, we studied some textural characteristics of patterns. It can be seen on Table 5, that the relative frequency of a species can varies among stripes to a relatively high (cf. *Grimmia pulvinata*) or less degree (*Deschampsia flexuosa*). It is remarkable, that the *variation coefficient* of taxon saturation (i.e. sum of relative frequencies) within the stripes is very low, so the

density of presences is rather similar in each stripe. Relative frequency of the plots containing given number of taxa varies within the stripes to relatively low degree (Fig. 4). The most frequent are those microquadrats in which just one taxon appears (50-60 %), while frequency of empty plots is equal to those that contain two populations (about 20 %).

Table 5. The relative frequency values of each taxon and all of them within the five stripes. The mean, the standard deviation and the variation coefficient of frequencies are also listed. For taxon names refer Table 1.

Tax.	*Stripe*					*Mean*	*Std.*	*Var.*
	A	*B*	*C*	*D*	*E*		*Dev.*	*Coeff.*
Pu	48.68	51.75	37.53	25.35	35.10	39.68	10.695	0.270
Ac	33.63	29.30	33.48	36.80	43.10	35.26	5.129	0.145
Gp	1.10	7.10	16.15	3.13	8.73	7.24	5.836	0.806
Df	7.10	5.03	5.15	6.35	3.88	5.50	1.252	0.228
Dc	3.18	4.10	3.20	4.63	11.15	5.25	3.355	0.639
Ra	2.10	3.75	4.78	6.30	3.35	4.06	1.579	0.389
Cp	2.53	2.13	3.63	5.20	2.83	3.26	1.216	0.373
Ep	1.98	1.05	0.98	1.90	2.03	1.59	0.525	0.331
Li	0.78	0.43	0.53	1.15	1.40	0.86	0.414	0.484
Hl	0.18	1.95	1.35	0.10	0.03	0.72	0.877	1.218
all	101.2	106.6	106.8	90.90	111.6	103.4	7.892	0.076

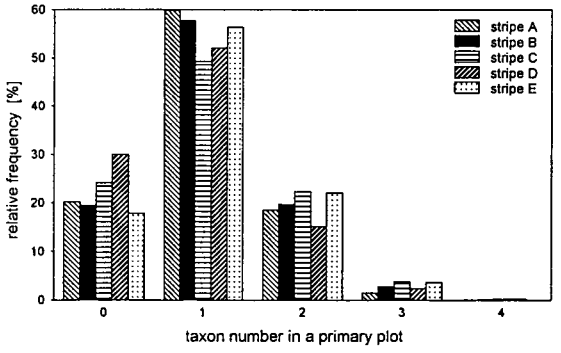

Fig. 4. Relative frequency histogram of the primary plots (microquadrats) containing given number of taxa in the five stripes.

The *fAss* curves of each stripes and whole sampling area are depicted on Fig. 5. An essential difference can be seen between the whole area and stripes. One reason of this deviation is that the *sample size were different* (cf. Table 2). (The effect of sample size can not be neglected even when the *fAss* values are standardized by it.) Larger sample size generates higher values for stripes in this case, but – because it was always the possible largest due to complete sampling procedure – it can not change the relative shape of an *fAss* curve. We can recognize however, that the curve for whole area is more flattened without any well expressed maximum value, compared to the average curve. Otherwise, the area of maximum *fAss* of average curve is rather similar to A_{ass} of whole area (0.36 m^2 and 0.49 m^2).

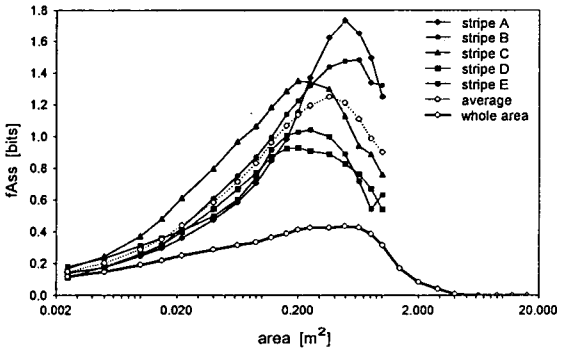

Fig. 5. The *fAss* curves of five stripes, their average, and whole sampling area.

Regarding to all *fAss* curves of stripes, both maximum value and its area are sufficiently variable. While *fAss* maximum is 0.928 bit in stripe D, in case of stripe A its value is nearly doubled (1.736 bit). Moreover, the A_{ass} varies between 0.2 m^2 and 0.64 m^2.

The *dAss* curves of stripes are also various, but theirs significant sections are more similar (Fig. 6). The smallest significant *dAss* maximum is 0.132 bit (in stripe D), and the largest one is 0.203 bit (in stripe C). The interval, in which the areas of *dAss* maximum of stripes are located, can be outlined between 0.01 m^2 and 0.0625 m^2. It is remarkable, that the average *dAss* curve of stripes is so much similar to curve of whole area, that the *dAss* maximum values are equal (0.14 bit), and the A_{ass} values, respectively (0.0225 m^2, cf. Fig. 6).

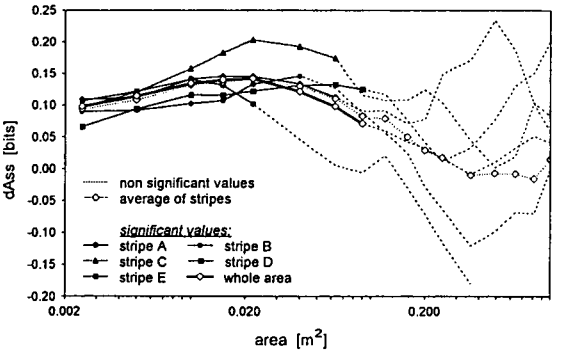

Fig. 6. The *dAss* curves of five stripes, their average, and whole sampling area. 99 random shifts were executed, $p = 0.01$.

For comparing different subtypes of associatum measures, we calculated the variation coefficient of functions along spatial scale, regarding to five stripes (Table 6). There are no considerable differences between *dAss* and cal*A*ss, while the *senAss* has a little bit greater values. Generally they have larger variation coefficient than the other three measures. The *fAss* and *relAss* are similar to one another, but the variation of *relAss* is smaller, *redAss* is represented by smaller coefficients in first several spatial series steps, but larger values in second half of spatial scale. (The curves are depicted on Fig. 8.)

Table 6. Variation coefficients of different associatum functions along spatial scale from five stripes. The coefficient has not been calculated when at least one associatum value was negative.

Area (m^2)	*fAss*	*redAss*	*relAss*	*dAss*	*calAss*	*senAss*
0.0025	0.1768	0.0285	0.1500	0.1852	0.2096	0.2201
0.0050	0.1722	0.0385	0.1282	0.1317	0.1389	0.1452
0.0100	0.1871	0.0527	0.1452	0.1666	0.1822	0.1899
0.0150	0.2120	0.0643	0.1739	0.2178	0.2220	0.2365
0.0220	0.2232	0.0847	0.1890	0.2698	0.2650	0.2869
0.0400	0.2215	0.1147	0.2002	0.4078	0.4023	0.4239
0.0630	0.2187	0.1557	0.2002	0.5704	0.5523	0.5728
0.0900	0.1747	0.1844	0.1534			
0.1200	0.1409	0.2307	0.1136			
0.1600	0.1360	0.2975	0.1117			
0.2000	0.1450	0.3656	0.1222			
0.2500	0.1737	0.4226	0.1608			
0.3600	0.2431	0.5544	0.2232			
0.4900	0.3198	0.5880	0.2932			
0.6400	0.3852	0.5389	0.3059			
0.8100	0.4210	0.4536	0.2986			
1.0000	0.4016	0.3475	0.2233			

Variability of associatum of blocks

As we continued the division of the stripes we have got altogether 20 blocks with 2.5 m^2 from the whole sampling area. Calculating *dAss* curve for each blocks, we can experience that the variability of spatial dependence increases if the sampling area decreases, as it was expected (Table 7). The relations among the different associatum measures in degree of variance are similar as they were noticeable in case of stripes.

Analysing the curves of *dAss* of blocks (Fig. 7), it can be seen that significant *dAss* values will appear more accidentally along the spatial scale. Some curves exhibit significant differences in neither spatial series step. Moreover, only 11 of the 20 curves have more than one significant values along the spatial scale. We can conclude the area of blocks being so small, that it can not represent perfectly the total sampling area, and the effects of a local pattern to the pattern organization can obscure the characteristics of entire community.

Table 7. Variation coefficients of different associatum functions along spatial scale from 20 blocks. The coefficient has not been calculated when at least one associatum value was negative.

Area (m^2)	*fAss*	*redAss*	*relAss*	*dAss*	*calAss*	*senAss*
0.0025	0.3689	0.0622	0.2960	0.5055	0.4572	0.4794
0.0050	0.3368	0.0691	0.2724	0.4933	0.4587	0.4761
0.0100	0.3308	0.0922	0.2717	0.5758	0.5359	0.5662
0.0150	0.3330	0.1137	0.2768	0.6878	0.6420	0.6844
0.0220	0.3259	0.1467	0.2659			
0.0400	0.3199	0.2040	0.2669			
0.0630	0.3450	0.2890	0.3000			
0.0900	0.3514	0.3695	0.3082			
0.1200	0.3491	0.4469	0.3100			
0.1600	0.3614	0.5198	0.3262			
0.2000	0.3800	0.5495	0.3349			
0.2500	0.3932	0.4955	0.3275			
0.3600	0.4824	0.3419	0.3596			
0.4900	0.6711	0.3766	0.4968			
0.6400	0.9034	0.3358	0.7263			
0.8100	1.1310	0.2985	1.0293			
1.0000	1.1453	0.2304	1.0586			

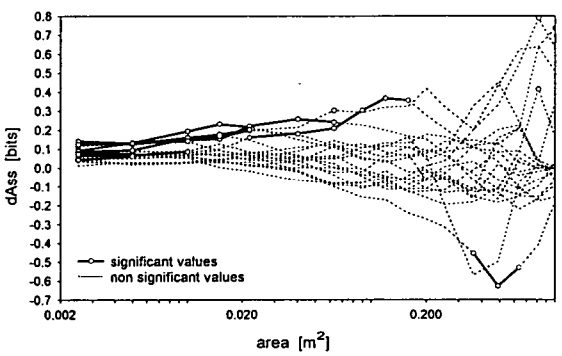

Fig. 7. The *dAss* curves of 20 blocks from field. Random reference was represented by 99 random shifts, $p = 0.01$.

We have revealed that each *dAss* curve of stripes and half of blocks have significant domains. They appear when we compare *fAss* curve of a parcel to its „own" random references. The next question is whether *dAss* (or other associatum) curves of all stripes (or blocks) can be significantly different from those which are originated from stripes of random patterns. In this case we have to study the overlap between a number of associatum curves from observed and random patterns, as we will explain in the next chapter.

Comparison of associatum curves from field data to those from random patterns

If the community of the whole sampling area is considered as a unit of vegetation (examining its spatial dependencies), and this unity does not damage when the grid is divided into parts, then we should

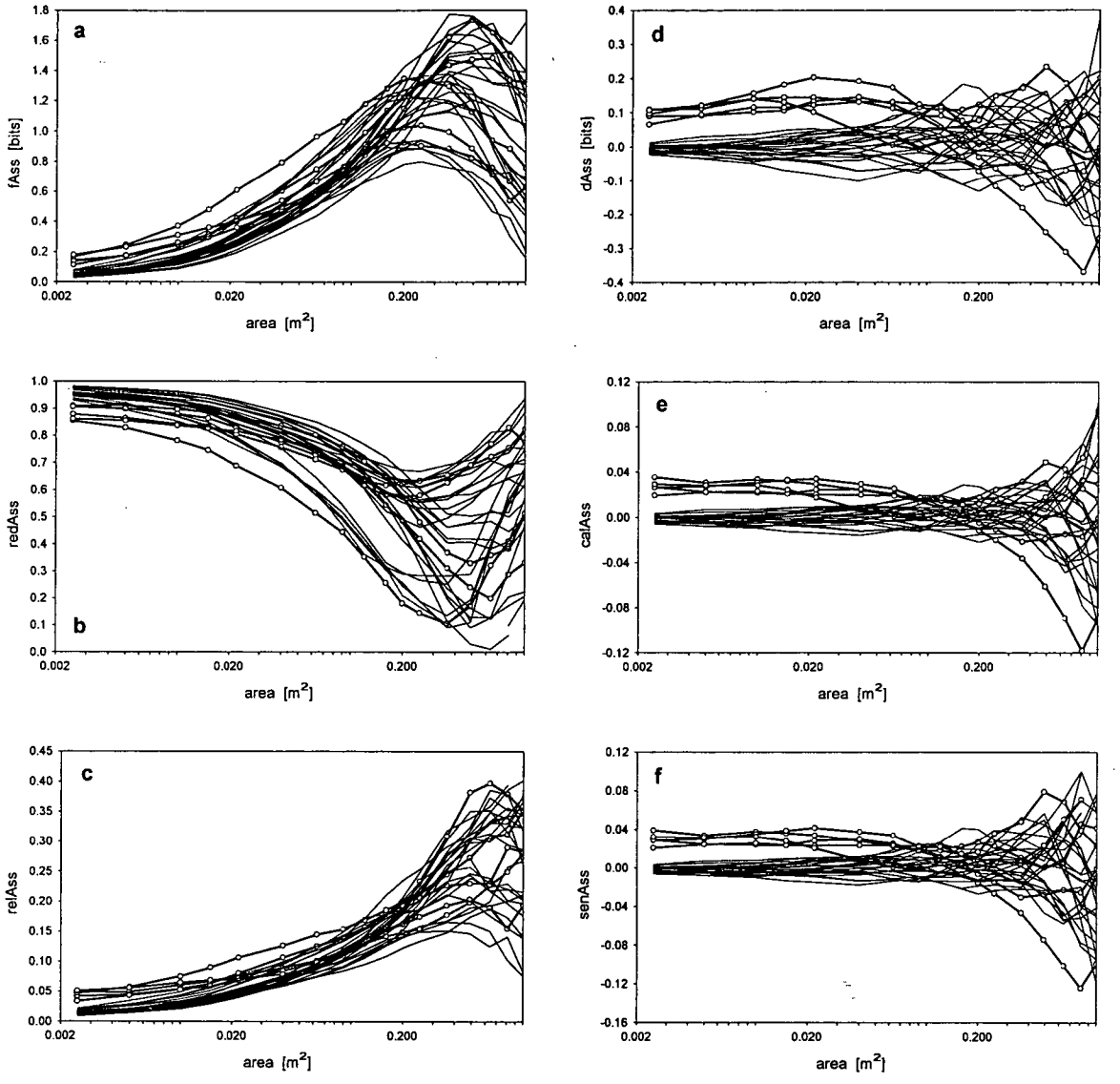

Fig. 8. The different associatum curves of 5 stripes from field pattern (thick lines with circles), and 25 stripes from 5 random patterns made by random shift (thin solid lines). For *dAss*, *calAss* and *senAss* 99 randomization were executed.

have to find that associatum curves of parts significantly differ from all curves calculated from parts of random patterns. The results explained above do not support the concept of unity, because both textural and structural measures exhibit considerable variability (cf. Table 5 and 6), and we have not had any information about associatum values of a null model yet. For solving this problem we made five random patterns applying random shift procedure, then designated the boundaries of five stripes in each random pattern (as we did it with field sample), and finally compared the five associatum curves from field to 25 other ones from random patterns (Fig. 8a-f). To detect any differences in median values, we applied *Mann-Whitney U-test* at each spatial series step (Table 8).

We can conclude that each applied measure of spatial dependence reveals significant difference from random patterns, but only in first half of spatial series steps. The significant interval of spatial scale however is shorter in case of *fAss*, *redAss* and *relAss*, than associatum functions using random references.

Moreover, while regarding to the second three functions (*dAss*, *calAss*, *senAss*), this interval corresponds to those in which the *dAss* itself also has the significant values (among them the maximum value, cf. Fig. 6), but in view of *fAss*, *redAss* and *relAss*, the maximum values appear outside of this significant interval (cf. Fig. 5). It means, that at characteristic associatum area we can find differences between field and random patterns if we use just the associatum difference (*dAss*) and its derived functions (*calAss* and *senAss*). The results show that when we divided the whole area into five stripes, the unity of community remained uncorrupted. So, each stripe with 10 m^2 can represent the 50 m^2 area of stand.

Table 8. Significance levels for Mann-Whitney test. The null hypothesis is that the median of associatum values from 5 field stripes is equal to the 25 ones calculated from strips of random patterns. The sign of significance levels: *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, while no marker indicates the non significant difference in a spatial series step.

Area (m^2)	*fAss*	*redAss*	*relAss*	*dAss*	*calAss*	*senAss*
0.0025	***	***	***	***	***	***
0.0050	***	***	***	***	***	***
0.0100	***	**	***	***	***	***
0.0150	**	**	***	***	***	***
0.0220	**	*	**	***	***	***
0.0400	*		*	**	**	**
0.0630				**	**	**
0.0900				*	*	*
0.1200				*	*	*
0.1600						
⋮						
1.0000						

To prove that this result is not an artifact, we compared the associatum curves of 5 stripes from one random pattern to other 4 × 5 ones (using *Mann-Whitney test*, as well). Since there are 5 random patterns and 17 spatial series steps, we applied the test 85 times at each associatum measure. From 85 comparisons, there were no significant differences regarding to *fAss*, *redAss*, *relAss*, and there were only two departures in case of *dAss*, *calAss* and *senAss* at $p \leq 0.05$.

Examining the consequences of further division, we compared the 20 field blocks to the other 20 blocks originated from one random pattern made by random shift. The results are listed in Table 9. Now, the block represent less the unity of community, and they are more different from each other, since significant deviation of associatum measures can be found only in first 2 – 5 spatial series steps.

Table 9. Significance levels for Mann-Whitney test. The null hypothesis is that the median of associatum values from 20 field blocks is equal to the 20 ones calculated from blocks of a random pattern. The sign of significance levels same as in Table 7.

Area (m^2)	*fAss*	*redAss*	*relAss*	*dAss*	*calAss*	*senAss*
0.0025	***	**	***	***	***	***
0.0050	***	*	***	***	***	***
0.0100	*		**	***	***	***
0.0150			*	***	***	***
0.0220				***	***	***
0.0400						
⋮						
1.0000						

Conclusions

(1) The random shift null model applied to *associatum difference* measure of large grid data have been validated. The probability of making the type I error is neither larger nor smaller than a chosen level of significance. In the validation procedure we used random shift not only for random patterns but to make pseudo-observed ones; this is in contradiction with Roxburgh and Matsuki (1999).

(2) The studied pioneer community exhibits low degree of mutual spatial dependence among populations. This pattern organization appears even when it is compared to random shift null model. It is noteworthy if we consider that the community consists of only ten taxa, and half of them are non vascular plants. Associatum values of the same order of magnitude were also detected in early stages of primary succession on dumps from open-cast coal mining in Hungary (Bartha 1990).

(3) The area of maximum value is 0.49 m^2 for *fAss*, but 0.0225 m^2 for *dAss* using random shift. This rather small (15 cm × 15 cm) area indicates, that we can find the community organizing effects among the interspecific interactions, and it is presumable, that the significant positive associatum is not a result of only spatial heterogeneity of abiotic conditions. The ordering of maximum areas gives the relation as $A_{flor} = A_{comp} < A_{ass}$. Regarding to *characteristic ordering*, *characteristic interval* and the concrete values of characteristic areas, the result is most similar to ten years old stand of study cited above (Bartha 1990). It seems that the pattern organization process takes place similarly in the different primary succession on clayey spoil banks, independently of floristic composition.

(4) The average of *dAss* curves from stripes is more similar to associatum curve from the whole sampling area than average of *fAss* values. It means

that the *dAss* originated from sufficiently large part of a stand can be more effective estimator of the characteristics of the „whole" community.

(5) The variability of the examined associatum curves can not be neglected, and it increases when the sampling area decreases. While the variation coefficients of *fAss*, *redAss* and *relAss* have slightly lower values than *dAss*, *calAss* and *senAss*, the last three measures can be regarded as the „better" ones, because in spite of this larger variability they can exhibit significantly different values from random patterns in broader interval of spatial scale. Moreover, in case of *dAss*, this significant interval contains the characteristic (maximum) value.

(6) Though the degree of variability is considerable, there is an interval of spatial scale at which the median of associatum values from particular field pattern significantly differs from that originated from random patterns. This fact, which was observable in stripes, can indicate a unity of pattern formation of vegetation. We can conclude that the 10 m^2 of sampling area represents the entire community. On the other hand, the unity begins to collapse if the sampling area is only 2.5 m^2. In the blocks, the significant *dAss* values appear rather accidentally corresponding to the local pattern. So, the sampling area with 2.5 m^2 can not be considered as an adequate size of pattern organization unit of the examined community.

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TISCIA 32, 31-34

HABITAT SELECTION OF ANT-TENDED APHIDS ON WILLOW TREES

N. Molnár, É. Kovács and L. Gallé

Molnár, N., Kovács, É. and Gallé, L. (2000): Habitat selection of ant-tended aphids on willow trees. - *Tiscia 32, 31-34.*

Abstract. We studied the spatial distribution of aphid populations as a function of host plant's properties and the ant species associated with them on dispersed willow trees (*Salix alba*) in the flood area of river Tisza. Our data based on the examination of 63 trees, 10 shoots on each. The presence of tending ants on willows promotes the maintenance of the aphid species diversity. The density of aphid populations were also positively influenced by the mutualists (mainly by *Lasius fuliginosus*). The relationships between the different aphid species and ants were various: *Chaitophorus vitellinae* showed stronger mutualistic interaction, than *Pterocomma* species. We have not experienced competitive interactions between aphid populations for services of ants. The different tree attributes had no significant influence on the habitat selection of aphids. Spatial distribution of aphids was affected by both the presence of ants and their number.

Keywords: aphids, ant-aphid mutualism, spatial distribution, ant-attendence, competition

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Introduction

In the mutualistic association of ants and aphids the main attractant is that aphids offer honeydew to ants, and ants provide them protection from natural enemies in return for this food source (Sudd 1987, Cushman and Beattie 1991). Host plant can influence this interaction, as the chemical composition and/or quantity of honeydew produced by herbivores varies with changes in host quality (Cushman 1991). The physiological state of host plants can play a role in determining the growth and size of aphid populations, too (Hales *et al.* 1997).

It is a well-known fact that an ant colony tends simultaneously several aphid species, thus there can be intra- or interspecific competition between aphid groups for the services of ants (Sudd 1987, Cushman and Addicott 1989, 1991, Dixon 1998).

According to Southwood and Kennedy (1983) trees are relatively large, structurally complex habitats, which have characteristic faunas within a species. The scattered willow trees could be regarded as microhabitats where the aphid and ant populations live more or less isolated from one another. The trees, however, provide different environmental conditions for aphids from several points of view. In this paper, we address the following questions: Which attributes of trees may play a significant role in the spatial distribution of aphids? Is there any role of ants in the survival of aphids having colonised randomly? Is there any difference between the aphid species in their mutualistic interactions with ants under these circumstances? Does the pattern of aphid populations indicate competition for the service of ants?

Methods

Aphid colonies were sampled from dispersed willow trees (*Salix alba*) in a flood plain of river Tisza in Kesznyéten Nature Reserve, NE-Hungary in May of 1998. May was convenient period because of great abundance of aphids and high activity of ants.

As distances between trees were relatively great (minimum distance between nearest trees was 3 m, maximum was 69 m), these trees could be regarded as microhabitats for aphids, characterised with size of trees and ant species living on them. We examined 63 willow trees, and 10 shoots on each.

Ant individuals staying on the tree trunk were counted for two minutes at each tree.

Trees were characterised with the distances to the nearest tree, the canopy area, trunk diameter and trunk height. We have computed correlation coefficients between the tree attributes and the occurrence of aphid species.

Results

We gathered approximately 15000 individuals of 5 aphid species and registered the presence of ants at the colonies. The most frequent aphid species were *Chaitophorus vitellinae* (Schrank), *Pterocomma rufipes* (Hartig) and *Pterocomma pilosum konoi* Hori (all three of the species are monoecious), while *Chaitophorus salijaponicus niger* Mordvilko and *Cavariella theobaldi* (Gillette and Bragg) were found in smaller quantity. Each aphid species were tended by ants but in variable degrees. Out of the 10 ant species found *Lasius fuliginosus* (Latreille) seemed to be the most important. Also *Lasius niger* (Linnaeus) and *Lasius brunneus* (Latreille) can play a role in attending aphids. The detailed analysis of ants basing on six years' field observation is given elsewhere (Gallé *et al.*, in prep.)

In the presence of attending ants there were more species of aphids per tree. The difference proved to be significant (by randomization test, $p<0.05$). We considered only those trees where aphids were found (59 trees).

Aphid species differed in their dependence on ants. Mutualism between *Chaitophorus vitellinae* and tending ants was stronger than that of *Pterocomma* species (Fig. 1). Mostly *Lasius fuliginosus* was responsible for these interactions (Fig. 2). The figures show that the individual number of *Ch. vitellinae* was strongly affected by the presence of ants. In the case of *P. rufipes* there is larger number of individuals in the absence of mutualists, however it indicates not a negative, but rather an indifferent interaction, as this species is also ant-attended.

In the presence of *L. fuliginosus* colony on the tree in question, more shoots were found colonised by aphids ($p<0.01$). Therefore, we can assume that the presence of *L. fuliginosus* is a crucial factor for aphids (Fig. 3)

No significant association was experienced between the different species of aphids occurring on the same tree according to $\chi 2$ test. Therefore, no competition can be assumed between the three most frequent aphid species for the services of ants.

The different attributes of willow trees (canopy area, trunk diameter and height) had no influence on the aphid species in selecting their habitats (Figs 4, 5, 6), since there is no detectable relationship between the habitat features and the percentage occurrence of the most frequent aphid populations. The most important habitat property was the density of ants (Fig. 7). These results are also demonstrated in Table 1.

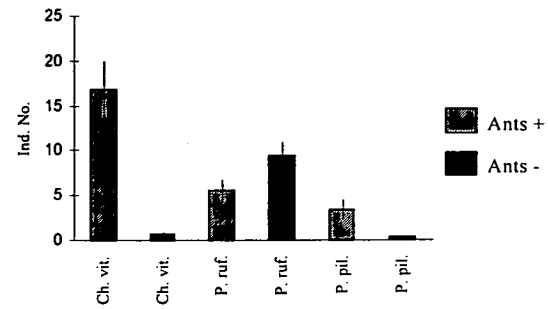

Fig. 1 Adult number of the most frequent aphid species in the presence and absence of attending ants.

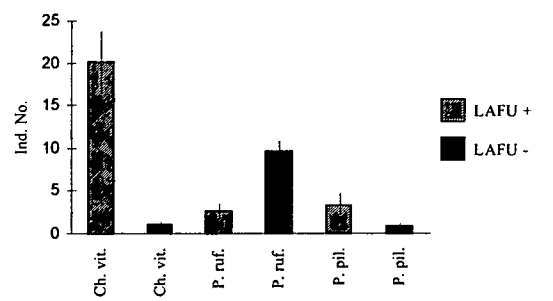

Fig. 2 Adult number of the most frequent aphid species in the presence and absence of *Lasius fuliginosus*.

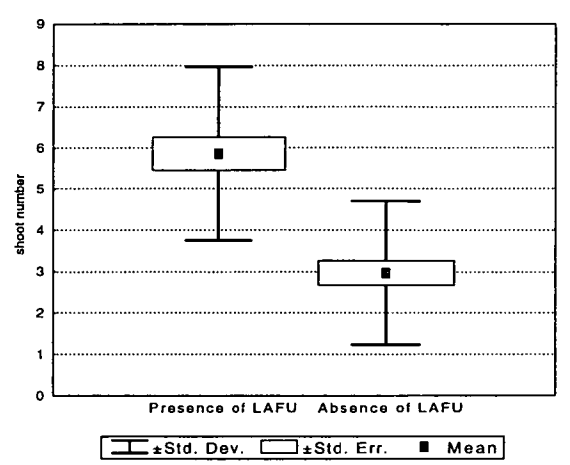

Fig. 3 Number of shoots per tree with aphids in the presence and absence of *Lasius fuliginosus*.

Significant correlation was found between the distances of the nearest trees and the trees' similarity based on aphid species composition ($r=-0.26$, $p<0.05$, $n=58$).

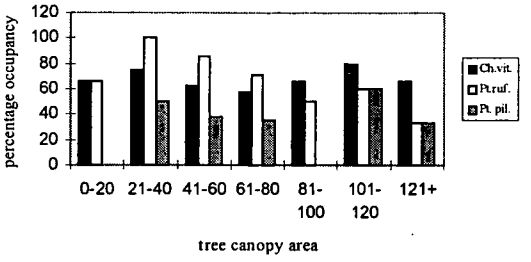

Fig. 4 Percentage occupancy of trees by the most frequent aphid species in tree canopy area (m^2) classes.

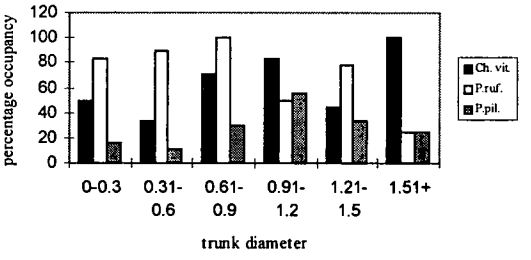

Fig. 5 Percentage occupancy of trees by the most frequent aphid species in tree trunk diameter (m) classes.

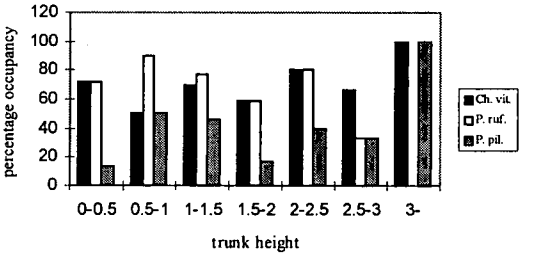

Fig. 6 Percentage occupancy of trees by the most frequent aphid species in tree trunk height (m) classes.

Discussion

Our study demonstrated that the presence of tending ants on willows promotes the maintenance of the aphid species diversity and population density. This is in accordance with the results of Fowler and MacGarvin (1985).

The number, distribution and activity of visiting ants are mainly regulated by the abundance and status of aphid populations and the peculiarities of aphid attendence vary with the ant species (Novgorodova and Reznikova 1996). According to an earlier investigation by Gallé *et al.* (1995) at the same site the habitat selection of ants depends on tree properties, such as inundation, trunk diameter and the density of aphids. Moreover, the distribution of ants is affected also by their interspecific competition, therefore they have mosaic-like distribution pattern.

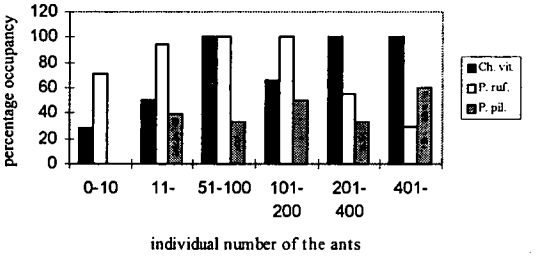

Fig. 7 Percentage occupancy of trees by the most frequent aphid species. Classes are established on the basis of individual number of the ants living on the willow trees.

Table 1 Correlation coefficients (Spearman Rank Correlation) with significancy levels between different tree attributes and the adult number of the most frequent aphid species.

	Ch. vitellinae	*P. rufipes*	*P. pilosum*
canopy area (n=59)	0.168 n.s.	-0.147 n.s.	-0.024 n.s.
trunk diameter (n=56)	0.209 n.s.	-0.192 n.s.	0.055 n.s.
trunk height (n=56)	0.200 n.s.	-0.101 n.s.	0.031 n.s.
ant individuals (n=63)	0.714 <0.001	-0.465 <0.01	0.368 <0.01

Mutualistic interactions are different in their strength and symmetry (Cushman and Addicott 1991). Aphids seem to be more dependent on ants than vice versa, because their distributional pattern on willows is determined only by ants, while ants' occurrence depends on other factors (e. g. competition), too. However, the aphid species differ in their dependence on the ants.

The role of host plants in aphid-ant mutualistic interactions was studied in different ways. According to Bristow (1991) the feeding site (floral or leaf tip) of aphids affects the ant attraction. The differential attractiveness probably reflects chemical differences in the honeydew. Skinner and Whittaker (1981) showed that the number of aphids increased with bud length, but this effect was far less important than that of ants. Cushman and Addicott (1989) revealed in their paper significant host impact on the *Aphis-Formica* mutualism if the analysis had involved the

presence or absence of ants, but their measure of host quality was rather crude: two levels of plant height (*Epilobium=Chamaenerion angustifolium*). The habitat selection of insect herbivores is affected by the host plant quality (Kareiva 1986). In the case of aphids the species and quantity of ants foraging on the herbivore's host plant also have a considerable importance.

The presence of aphids and the aphids' density on certain trees seem to depend on ant colonies living on the willows.

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TISCIA 32, 35-42

HABITAT CORRELATES OF ANT ASSEMBLAGES IN DIFFERENT FORESTS OF THE SOUTH PANNONIAN PLAIN

M. Alvarado

Alvarado, M. (2000): Habitat correlates of ant assemblages in different forests of the South Pannonian Plain. —*Tiscia 32, 35-42*

Abstract. Ant assemblages and their potential habitat correlates were studied in 20 forests of different tree composition (oak, poplar, juniper, black locust, pine, Russian olive and hybrid poplar), age, state of isolation and anthropogenic disturbance. The forests were characterized with 148 habitat scores, grouped in five: vegetation architecture, vegetation composition, microclimate, soil properties and the number, the condition and the size of the twigs on the ground surface. A non-parametric correlation analysis was carried out between the configuration of the observed habitats in principal coordinate factor spaces obtained on the basis of their ant assemblage composition and the different groups of habitat scores. Significant correlation was revealed between ant assemblages and vegetation architecture, vegetation composition and the twigs on the ground surface. Another computation of correlation between single habitat scores and the axes of PCoA factor space of ant assemblages showed that ants correlated with vegetation cover at 5 cm height, with the average tree diameter, with all the measured parameters of twigs and with the soil pH.

Key words: ant communities, native and planted forests, habitat conditions

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Introduction

One of the classical approaches of community ecology is to seek for the relations between the composition of ecological assemblages and the properties of their habitats. The roots of such studies are traced back to the "meteorological theories", which regard physical habitat attributes, such as microclimate or soil characteristics to be responsible for the presence, density and regulation of the populations or communities in question. In ants, this kind of approach was employed by several authors (e.g. Gallé 1972a, 1972b, 1975, 1979, Doncaster 1981, Johnson 1992), emphasizing the ultimate role of soil and microclimate in structuring the ant communities. Later, however, the habitat properties were considered only as one subset of the possible external correlates of ant assemblages. Besides physical factors, the food supply is often found to be effective in structuring ant communities, as well (Brian 1964, Andersen 1983, Marsh 1986, Seifert 1986, Sommer and Cagniant 1988). The role of vegetation, especially its architecture was investigated by several authors (Majer 1972, Greenslade and Greenslade 1977, Andersen 1992, Huxley and Cutler 1991) and manifold community-level interactions were established between ants and other animals, such as birds (Haemig 1992), mammals and birds (Kelrick *et al.* 1986), aphids (Stary 1987, Molnár *et al.* 1999, Reznikova and Novgorodova 1999), the epigeic and herb layer fauna (Gallé 1991, Gallé *et al.* 1998).

In our previous paper (Alvarado and Gallé 1999) we reported the distribution of ants in Hungarian lowland forests of different tree composition and different level of disturbance. In this paper we concentrate on habitat properties (plant species composition, architecture and degradation tolerance spectrum of the vegetation, microclimate, the number and condition of dead twigs on the ground surface and soil properties) as possible external correlates of ant assemblages in the same set of habitats.

Table 1. Attributes used for the characterization of the studied forest sites

Group	Attributes	No. of categories
1. Vegetation composition	1.1 Relative frequency of predominant plant species	97
2. Vegetation architecture	2.1 Whole vegetation cover	1
	2.2 Litter cover	1
	2.3 Litter thickness	1
	2.4 Vegetation cover at 15, 30, 70, 150, 300 cm	5
	2.5 Canopy cover	1
	2.6 Cover of mosses and lichens	1
	2.7 Tree diameter average	1
3. Microclimate	3.1 Temperature of soil and of air above soil surface, above herb layer, above 30 cm and at 1.6 m.	4
	3.2 Humidity of soil surface, herb layer, above 30 cm and at 1.6 m	5
4. Dead twigs on the ground surface	4.1 Density of dead branches of various sizes and conditions	20
5. Soil	5.1 Soil humidity	3
	5.2 Soil hygroscopic values	7
	5.3 pH of soil	1
Total		148

Material and methods

Habitat types

Research was conducted in twenty sites in the South Hungarian Plain, near Szeged, approximately 200 km south-east of Budapest. The sites selected for the study included indigenous and introduced forests, and we made a note of their location, complexity, isolation and degree of anthropogenic disturbance (Table 1). They were located: on the outskirts of Szeged (3 patches); in Ópusztaszer (30 km north of Szeged, 3 patches); in Balástya (35 km north-west of Szeged, 3 patches); in Ásotthalom (25 km west of Szeged, 9 patches); in Bócsa and Bugac regions of the Kiskunság National Park (50 km north-west of Szeged, 2 patches).

Ant sampling

Ants were sampled with pitfall traps. Traps were 6 cm diameter plastic cups, partly filled with ethylene-glycol which acted as a preservative. 3 × 5 traps at least with 3 m spacing were employed at each site. The traps worked for three consecutive 7-day periods, in May, June and July. The ants were preserved in 70% ethanol and identified by species level using standard keys and more recent reviews (Seifert 1988a, 1988b, 1992, 1996)

Habitat attributes

We used 148 habitat scores for a detailed characterization of the sites and the scores were grouped in five: vegetation composition, vegetation architecture, microclimate, number and condition of dead twigs on the ground surface and soil properties (Table 1).

The vegetation composition was established by assessing the relative frequency (percentage cover values) of the predominant plant species. One attribute group consisting of 97 scores (i.e. plant species) was obtained from this assessment. Plants were classified according to their degradation tolerance by Németh's evaluation system (Horváth *et al.* 1995). Since the plant species observed in the forests in question, belonged to three out of Németh's five categories: (1) moderately degradation-tolerant species, (2) degradation tolerant species, and (3) degradophilous species, it would tell that the sampled habitats are exposed to man-induced disturbances. Therefore we classified the sampled habitats on the basis of the assessed rate of disturbance. The disturbance degree was based on the amount of human influence or anthropogenic disturbance evident at each site. For this aim, we created a scale of four categories: nature reserve without disturbance (0); plantation with slight forester's activity (1); apparent tourist traffic, litter, etc. (2); grazing, repeated cuttings (3). The age, degree of disturbance and vegetation of the studied forests are summarized in Table 1.

In the analysis of the vegetation architecture, the total coverage values of the litter, soil, mosses, lichens and those of the higher plants at different heights (5, 15, 30, 50, 75 cm) were assessed and expressed as percentages. 12 scores, belonging to five attribute classes were obtained in this way (Table 2).

In the microclimate analysis the temperature and humidity were measured every month at each site (four times per site). Two groups containing

Table 2. Location, age, isolation, and the degree of disturbance of 20 studied forests in the South Hungarian Plain

Number	Location	Forest type	Age (years)	Disturbance	Isolation
1	Ásotthalom	poplar	100	0	medium
2	Ásotthalom	poplar	100	0	medium
3	Bugac	poplar	40	0	high
4	Ásotthalom	oak	80	2	medium
5	Ásotthalom	oak	80	1	medium
6	Bugac	oak	40	0	high
7	Ópusztaszer	oak	60	3	low
8	Ópusztaszer	oak	60	3	low
9	Bugac	juniper	30	0	high
10	Ásotthalom	black locust	50	3	low
11	Ópusztaszer	black locust	30	2	low
12	Bugac	black locust	15	0	high
13	Ásotthalom	pine	40	1	medium
14	Ásotthalom	pine	25	1	medium
15	Ásotthalom	pine	15	1	medium
16	Ásotthalom	pine	5	1	medium
17	Szeged	Russian olive	15	3	low
18	Balástya	hybrid poplar	30	2	low
19	Balástya	hybrid poplar	15	2	low
20	Balástya	hybrid poplar	15	2	low

altogether 9 categories were measured and recorded (Table 2). The soil temperature was measured at a depth of 5 cm and on the soil surface. The air temperature was measured at 30 cm and 160 cm above the soil surface. As the simultaneous measurement of all 20 places would have been impossible, data was taken from meteorological stations and used as standard reference.

The average number of dead twigs on the ground surface was estimated in 50 by 50 cm quadrates. The size and the condition (dry, fresh, rotten, only bark) of each twig were also noted. A total sample of fifteen such quadrates were taken at each site and 5819 twigs were measured in total. From the data we found oak forests having the highest, while juniper and Russian olive forests had the lowest number of twigs.

From among the soil properties, soil water content was measured every month, hygroscopic values and soil pH were also analyzed by standard methods. Three attribute groups and altogether 11 scores were recorded.

Data analysis

Since the most of our data were not of normal distribution, we employed non-parametric statistics in the majority of cases.

A principal coordinate analysis (PCoA) was performed on the basis of ant assemblage composition and each set of the above-mentioned habitat scores (five in total). NuCoSa (Tóthmérész 1993) and Syn-Tax packages (Podani 1995) were used for the computations. The resemblance matrices were set up using Sørensen's presence-absence index for the vegetation composition and Czekanowski's index for the other score groups and for ant assemblages. In order to identify the groups of external factors (i.e. core groups) that are correlated with, and therefore presumably affect the habitat differentiation of ant assemblages, we computed Spearman's rank correlation between PCoA factor spaces made on the basis of ants and habitat score groups. For the correlation analysis, the relative position of the forest sites in the PCoA spaces (i.e. the multidimensional Euclidean distances between the points representing the sites) were measured and correlated. In order to reveal the role of single factors, also Spearman's rank correlation was calculated between the PCoA axes of ant assemblages and each single factor. Successive rank correlation was computed to look for a possible statistical link between the relative frequency of ant species and the habitat scores. Most analyses produced composed statistical tables, therefore the significance values were corrected with Bonferroni's test (Bonferroni 1935, 1936).

Results

Composition of ant assemblages

Altogether 36 ant species were recorded in the sampled forests (Table 3, see also Alvarado and Gallé 1999). The majority of them are typical forests species (e.g. *Leptothorax, Myrmica* spp., *Dolichoderus quadripunctatus, Camponotus* spp., with exception of *C. piceus, Formica fusca* and the

mound-building *Formica* s. str. species etc.), but some (e.g. *Anergates atratulus, Camponotus piceus, Cataglyphis aenescens, Formica cunicularia, F. rufibarbis, Lasius niger, L. alienus, L. psammophilus, L. carniolicus, Myrmica schencki, Plagiolepis vindobonensis, Polyergus rufescens, Solenopsis fugax, Tapinoma ambiguum*) are more characteristical for grassland habitats in the studied region (Gallé 1972)

Table 3. List of ant species recorded in different forests in the South Hungarian Plain.

No.	Species
	Subfamily: Myrmicinae
1	*Anergates atratulus* (Schenck, 1852)
2	*Leptothorax nylanderi* (Förster, 1850)
3	*Leptothorax interruptus* (Schenck, 1852)
4	*Leptothorax unifasciatus* (Latreille 1798)
5	*Myrmica rubra* (Linnaeus, 1758)
6	*Myrmica sabuleti* Meinert, 1861
7	*Myrmica salina* Ruzsky, 1905
8	*Myrmica schencki* Emery, 1895
9	*Myrmecina graminicola* (Latreille, 1802)
10	*Solenopsis fugax* (Latreille, 1798)
11	*Tetramorium caespitum* (Linnaeus, 1758)
	Subfamily: Dolichoderinae
12	*Dolichoderus quadripunctatus* (Linnaeus, 1758))
13	*Tapinoma ambiguum* Emery, 1925
	Subfamily: Formicinae
14	*Camponotus fallax* (Nylander, 1850)
15	*Camponotus piceus* (Leach, 1825)
16	*Camponotus truncatus* (Spinola, 1808)
17	*Camponotus vagus* (Scopoli, 1763)
18	*Cataglyphis aenescens* (Nylander, 1849)
19	*Cthonolasius* sp.
20	*Formica cunicularia* Latreille, 1798
21	*Formica fusca* Linnaeus, 1758
22	*Formica polyctena* Förster, 1850
23	*Formica pratensis* Retzius, 1783
24	*Formica rufa* Linnaeus, 1761
25	*Formica rufibarbis* Fabricius, 1793
26	*Formica sanguinea* Latreille, 1798
27	*Formica truncorum* Fabricius, 1804
28	*Plagiolepis vindobonensis* Lomnicki, 1925
29	*Polyergus rufescens* (Latreille, 1798)
30	*Lasius alienus* (Förster, 1850)
31	*Lasius fuliginosus* (Latreille, 1798)
32	*Lasius niger* (Linnaeus, 1758)
33	*Lasius paralienus* Seifert, 1992
34	*Lasius platythorax* Seifert, 1992
35	*Lasius psammophilus* Seifert, 1992
36	*Lasius carniolicus* Mayr, 1861

Correlation with habitat attributes

A significant rank correlation was found between PCoA space of the ant assemblages and that of the vegetation architecture, the vegetation composition and the twigs on the ground surface, respectively. No significant correlation was noticed, however, with microclimate and soil score groups (Table 4).

Table 4. Spearman's rank correlation between PCoA spaces of different groups of the habitat cores and that of the ant assemblages. |r| = absolute value of correlation coefficient; p = level of significance already corrected with Bonferroni's test

Score groups	|r|	p
Vegetation architecture	0.361	<<0.001
Vegetation composition	0.258	0.016
Microclimate	0.124	n.s.
Dead twigs on the ground surface	0.439	<<0.001
Soil	0.005	n.s.

At the Spearman rank correlation between the first three PCoA coordinates of the ant assemblages and the various habitat scores mentioned, we found that the first coordinate correlated significantly with the number and condition of twigs on the ground surface, vegetation cover at 30 cm, percentage of mosses and lichens, and the tree diameter average measured at each place (Table 5). The second axis correlated with vegetation composition (i.e. the number of plant species present at each site) and vegetation cover at 5 and at 15 cm. The third coordinate correlated with the soil pH.

Besides these habitat characteristics, there was a close correlation between the second axis of the above PCoA factor space and the degree of anthropogenic disturbance of the sampled habitats ($r = 0.58$, $p<0.01$).

The following correlation was established between the frequency of the ant species and the above-mentioned habitat scores (Table 6). The frequency of both grassland (*L. alienus, L. niger, S. fugax, F. rufibarbis*) and open forest species (*C. vagus, F. fusca*) correlated with the cover of herb layer either at 5 or at 15 cm above the ground. Grassland species showed negative correlation with tree diameter, while *F. fusca*, which is one of the most typical forest species in this region, had a positive correlation. Only the frequency of *L. niger* and *Leptothorax unifasciatus* was in correlation with the litter cover, in the case of the number of twigs, however, the grassland and forest species are well separated on the basis of their correlation. *Lasius platythorax* was the only species that correlated significantly with the pH of the soil.

Discussion

In our attempt to reveal which habitat conditions are correlated with the composition of ant assemblages, we found that vegetation composition, vegetation architecture, and the number and condition of dead twigs on the ground surface presumably affect the distribution and diversity of ants. In addition to differentiation among forest types, the degree of differentiation according to

Table 5. Factors correlated with axes of PCoA ordination space of the different forest on the basis of the composition of ant assemblages. |r| = absolute value of correlation coefficient; p = level of significance; p(corr) level of significance corrected with Bonferroni test

Factors	axis	|r|	p	p(corr)
Vegetation cover at 5 cm	II	0.565	0.009	0.072
Vegetation cover at 15 cm	II	0.481	0.032	0.256
Vegetation cover at 30 cm	I	0.474	0.035	0.280
Cover of mosses and lichens	I	0.462	0.040	0.320
Tree diameter	I	0.625	0.003	0.024
Abundance of different plant species	II	0.522(?)	0.018	0.144
No of dead twigs	I	0.607	0.005	0.040
Soil pH	III	0.594	0.007	0.048

Table 6. Spearman's rank correlation between the relative frequency of ant species and habitat attributes (significant values only)

Attributes	Ant species	r	p
Litter cover	*Lasius niger*	0.45	0.08
	Leptothorax unifasciatus	0.49	0.02
Vegetation cover at 5 cm	*Lasius alienus*	0.45	0.04
	Solenopsis fugax	0.65	0.002
	Formica rufibarbis	0.62	0.006
	Lasius niger	0.45	0.08
	Camponotus vagus	0.50	0.02
Vegetation cover at 30 cm	*Myrmica sabuleti*	0.49	0.02
	Formica fusca	0.51	0.04
	Camponotus vagus	0.72	0.0003
Tree diameter average	*Lasius paralienus*	-0.48	0.03
	Tetramorium caespitum	-0.49	0.04
	Tapinoma ambiguum	-0.48	0.06
	Formica fusca	0.47	0.072
Number of twigs	*Tetramorium caespitum*	-0.56	0.02
	Tapinoma ambiguum	-0.58	0.012
	Solenopsis fugax	-0.50	0.04
	Formica rufibarbis	-0.49	0.04
	Leptothorax nylanderi.	0.76	0.0001
	Dolichoderus quadripunctatus	0.64	0.002
	Camponotus fallax	0.68	0.0009
pH of soil	*Lasius platythorax*	-0.52	0.01

"microsite" variation (Whittaker and Levin 1977) within forest types is important. It is not an "average forest" that most species require but some particular elements within the forest itself.

Brian (1964) was one of the first to establish that since ants are not randomly distributed but have acquired some relation to the pattern of habitat variation it must be presumed that two obvious mechanisms of assortment exist: the first is that queens must have some power to select the best habitat in which to live, while the second is that the colonies once established may further assort themselves through population pressure largely dependent on the success with which they exploit their immediate resources and their potentiality for aggression. He also learned from the results of PCA analysis that moisture, the nutrient status and wind were the most important factors for heathland ants.

Perfecto and Vandermeer (1996) found that shade and leaf litter had a significant effect on the ant fauna in tropical agroecosystems but also probably (for indirect reasons) having to do with species interactions. Parameters belonging to the vegetation architecture are factors that are intertwined and operate together to provide ants with food and good nesting sites. Gallé and coworkers (Gallé 1991, Gallé *et al.* 1992, 1998), working in different successive stages of sand-dune areas in Southern Finland, Hungary, Poland, and Turkey, found that the vegetation architecture, the number and condition of the dead twigs on the ground surface and the composition of epigeic invertebrate fauna are assumed to be of importance in differentiating the composition of ant communities. Under more extreme climatic conditions the microclimate was also correlated with ant community composition (Járdán *et al.* 1993), but in our case no correlation was seen between microclimate and the composition of ant assemblages. The reason could be that no

microclimatic extremities occur in these forests because of the shading effect produced by the canopies.

The importance of small-scale heterogeneity and variation in diversity in forest-floor invertebrates is also a very important aspect to consider and has been well documented by Niemelä *et al.* (1996). They found that a major element creating systematic variation in local assemblages is the contrast between mature and young forests. Both forest physiognomy, through its effect on microclimate, and the composition of undergrowth, through its effect on micro-habitats and litter, are presumably important determinants of this difference. Andersen (1986) established that a greater structural complexity of vegetation will increase the availability of nesting and foraging sites, the level of ground insolation, and possibly food supply as well. The complexity of vegetation architecture is one of the most important component of the habitat heterogeneity that can be responsible for the increasing diversity in ant community succession (Kondoh and Kitazawa 1984, Gallé 1991, Járdán *et al.* 1993, Gallé *et al.* 1998). A positive correlation generally exists between structural diversity of the habitat, and the abundance and diversity of ants (Culver 1974, Room 1975, Greenslade and Halliday 1983). The soil-litter complex have also been documented by Andersen (1986) as a prime parameter for cryptic predators and scavenger ants in the soil.

Seifert (1986) described the environmental background of different ant communities on the basis of plant species composition and cover in different layers. We saw that plant species composition influences ant assemblages, depending on the kind of plant taxons present in the different sites. More degraded places showed lower values of ant diversity and species richness. The site differences in species composition could then match the differences in vegetation. This is consistent with the widespread finding that distinctive ant assemblages are often associated with different vegetation types (Brian 1964, Brown 1959, Cole 1934, Gallé *et al.* 1985, Greenslade 1971, Greenslade and Greenslade 1977, Hayashida 1960, Majer 1972, Markó 1999, Room 1971, Sanders 1970, Talbot 1934, Yasuno 1963, but see Markó 1998). Mabelis, as early as 1977 established three important factors that influence ants: amount of shade, soil moisture and number of plant species. The number of plant species plays an important role in supporting plant lice which are in close connection with ant species and also offer an abundance of items for predation. More plant species means a broader spectrum of different animals as well as more diverse nesting places for the ants.

In the case of soil the only parameter that had a significant correlation was the acidity-alkalinity of the soil. The explanation is not very easy, because ants as constructing their nests have an effect on changes in soil processes, e.g. a buffering the soil pH, therefore cannot expect a strong dependence of ant populations on the pH of the soil. The effect could be reverse: the ant populations differently modify the pH of the soil, therefore this correlation could be observed. Ants modify the abundance of different microflora groups and these changes depend on the size of ant colonies and on their biomass turnover. The size of a colony generally has an inhibiting effect on the abundance of ammonifying bacteria and stimulates the abundance of *Actinomycetes*. The turnover of biomass has a positive effect on the number of ammonifying bacteria. In this connection ant constructing species have different effects on the pH and the content of exchangeable cations in the soil of nests (Petal 1980). Ant mounds also provide an environment for plants, one that has better aeration and is warmer as well as nutrient-rich (Lesica 1998). Compared with surrounding soils, ant-hills often have a significantly higher pH and cation content, which suggests that ant activities counteract leaching (King 1981). *Lasius platythorax* nests are known because they contains several internal basic elements and fungi hyphae (Seifert 1996). Therefore, this modifying role of ant nests on the soil properties could explain the high significant correlation ($p=0.01$) between *L. platythorax* and pH.

A comparison of the results presented in this article with the previous one suggests that vegetation architecture (vegetation cover at different heights, litter cover, percentage of mosses and lichens, and tree diameter), vegetation composition and the number and condition of twigs on the ground surface are the main habitat attributes which most likely play a role in structuring ant assemblages in the investigated forest patches. We think that these habitat attributes are the primary ones that count for ants in the process of choosing a place to establish themselves. After ants find a definite site, interspecific competition for food, nests and territories will also help to structure the ant assemblages and determine the way in which they interact with other ant species. Decaying wood, leaf litter, tree trunks and the presence of other plant species provide a variety of microhabitats that most probably disappear with the intensification of forestry management and dominance of exotic trees.

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TISCIA 32, 43-46

NOTES ON THE COEXISTENCE OF THE SUPERCOLONIAL *LASIUS NEGLECTUS* VAN LOON, BOOMSMA ET ANDRÁSFALVY 1990 (HYMENOPTERA: FORMICIDAE) WITH OTHER ANT SPECIES

A. Tartally

Tartally, A. (2000): Notes on the coexistence of the supercolonial *Lasius neglectus* van Loon, Boomsma et Andrásfalvy 1990 (Hymenoptera: Formicidae) with other ant species. — *Tiscia 32, 43-46.*

Abstract. *Lasius neglectus* is an invasive species known for about ten years. This species excludes other ant species from the areas of its supercolonies and causes much trouble for people by intruding to their houses. Apart from these, there is not much we know about the ecology of *L. neglectus*. The aim of my paper is to compare ant communities living in the border and in the centre of the *L. neglectus* colonies. As my experiences show *L. neglectus* reaches a quite high abundance in the centres of the supercolonies at the expense of the abundance of other ant species. It was proved using the χ^2 test, that the distribution of the *L. neglectus* in the centre area and the border was significantly different ($p<0.95$). We have also demonstrated that the Shannon diversity of the centre assemblage is significantly smaller than the diversity of the border ($p<0.95$). This can be explained by the polygynous strategy, which is characteristic for *Lasius neglectus*.

Keywords: Lasius neglectus, *supercolony, colony formation, ant communities, coexistence, aggression*

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Introduction

Lasius neglectus van Loon, Boomsma et Andrásfalvy, 1990 was described as an invasive species in Hungary (van Loon *et al.* 1990). Former field studies clearly show that deeper ecological knowledge of this species could be important in many respects. Presumably this is an invasive species of the Hungarian fauna, which is able to colonise many different habitats and successfully excludes the indigenous ant species from large areas (van Loon *et al.* 1990, Tartally 1999, 2000). It is also worth to mention that most of its supercolonies are in human settlements. The huge amount of *L. neglectus* cause a lot of inconveniences for people living in infected houses. The fact that in 1998 the species was described of a 2 km^2 supercolony (van Loon *et al.* 1990). In that time this species was known in three localities in Budapest. Since then the number and the size of known Hungarian supercolonies increased (Tartally 1999, 2000) and supercolonies from Spain (Espadaler 1999), from Rumania (Markó 1998) and Czechia by Seifert (personal comm. by S. Csősz), have been recorded. *L. neglectus* has an ecological plasticity, it is able to successfully colonise in different wooded and unwooded habitats, and 29.7% of the Hungarian ant fauna (Gallé *et al.* 1998) was found on the edge of *L. neglectus* supercolony areas (Tartally 1999, 2000). Colonies of *L. neglectus* contain several queens. Another polygynous species, the *L. sakagamii* Yamauchi and Hayashida 1970, known in the *Lasius* subgenus, occurs in Japan (Yamauchi and Hayashida 1970, Yamaichi *et al.* 1981).

The publications so far on *L. neglectus* (van Loon *et al.* 1990, Boomsma *et al.* 1990a, 1990b, Seifert 1992, 1996, Gallé *et al.* 1998, Markó 1998, Espadaler 1999) are concerned with the taxonomy and distribution. The aim of this paper is to compare ant assemblages living in the border and in the centre of the *L. neglectus* colonies.

Materials and methods

A supercolony of *L. neglectus* was studied (Tartally 1999, 2000), in the Botanical Garden of Kossuth Lajos University, Debrecen, Hungary in 1998. The area of this colony is 0.07 km^2. This is the most natural area in Hungary, where *L. neglectus* supercolony has been found. Grassland patches and planted trees (*Thuja occidentalis, Juniperus virginiana, J. communis, Chamaecyparis lawsoniana, C. pisifera, Buxus sempervirens, Picea abies, Pinus strobus, P. mugo, Sorbus aucuparia, S. borbásii, Acer pseudoplatanus, Fraxinus excelsior, Abies concolor, Magnolia kobus, Pyrus pyraster, Quercus robur*) alternate in the area. The dendrology map of the garden is given by Papp (1997). Three types of sites were differentiated:

(1) The area where the workers venture out during food searching is the border of the colony. (2) Where the mosaic of *L. neglectus* and other ant species nests alternates is called the edge of the colony. (3) The centre of the colony is the area rarely occupied by other ant species nests.

No aggressive behaviour was observed between the workers from the different parts of the site, therefore I regarded all the nests to belong to the same supercolony.

On the area of the supercolony in Debrecen I did the following assays:

(1) I chose two 5×5 m grassy areas, on the edge and in the centre of the colony, where the nests of various ant species were mapped with an 1×1 m frame divided by 10×10 cm squares. The squares contained at least one exit we marked with different characters depending on the species (Fig. 1a, b). If the owner species of the exit was not active, I dig till I found workers. The 10×10 cm squares were reasonable choices because no any square contained exits of different species.

The distribution of the *L. neglectus* in the centre area and the border was compared by the $\div^2$ test. The diversity of the ant assemblages of these areas was compared by the Hutcheson test of the Shannon diversities using the NuCoSA package (Tóthmérész 1993).

(2) The centre of the colony is crossed by a road with flower-beds on both sides, considered as a homogeneous habitat. A transect line along the flower-beds, consisted of 34 sample points (one in every five meter) was employed through the colony. It was 170 meters long. I used the following sampling methods on the transect:

(a) The transect was divided 5 meter long sections with the sampling points in the middle and I counted the numbers of 10 cm long parts of the sections between the road and the kerb (which is 2 centimetres wide) where the exits of the ant species were found (Fig 2a).

(b) Pitfall traps were settled on the sampling points for ten days. I used 3 cm in diameter boxes filled with ethylene glycol. I counted the numbers of individuals from all the different ant species that fell into the trap.

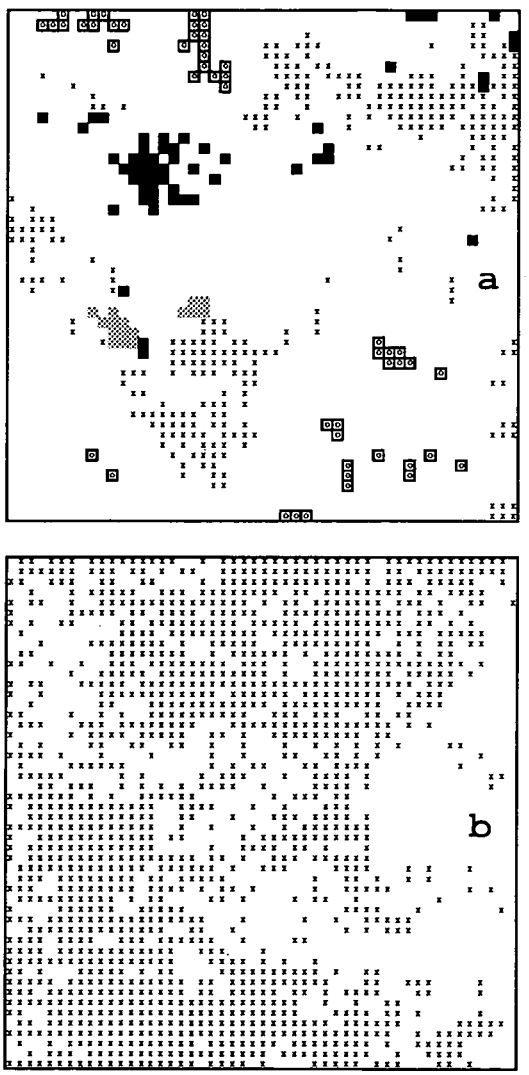

Fig. 1. 5 x 5 m areas, one in the edge of the colony (a), and in the centre (b). These represent 10 x 10 cm areas by characters that include exits of certain species. X: *Lasius neglectus*; O : *Lasius niger*; ■: *Polyergus rufescens*; ■: *Tetramorium caespitum*.

(c) Baits also were placed on the sampling points on 14. 6. 1998. at four o'clock. Thirty minutes later I estimated the number of individuals on the baits. As baits I used discs with the diameter of 7.7

cm fixed with a nail to the ground, in the middle honey and meat paste were placed. Only the number of ants right on the discs were estimated, I ignored the other individuals around the bait discs.

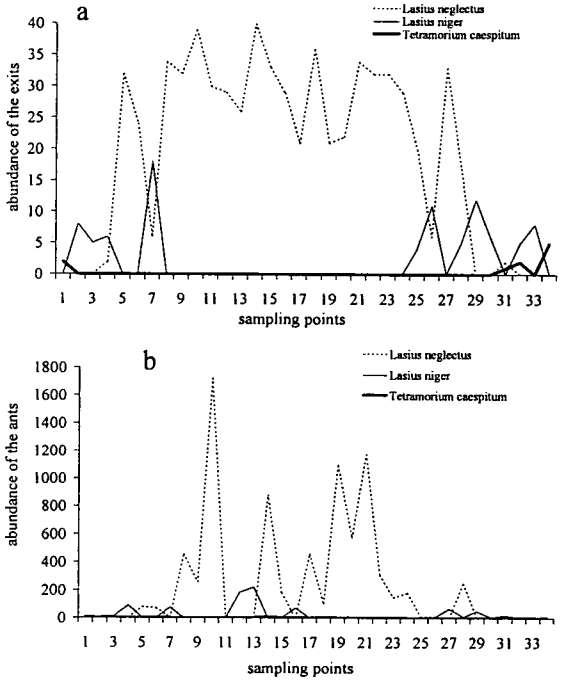

Fig. 2. The abundance of different ant species abundance on the basis of exits counted (a), pitfall traps (b) and baits (c).in the sampling points of the transect (170 m) crossing the *Lasius neglectus* supercolony

Results

At the edge of the colony exits of *L. neglectus* have been found, among other ant species' exits such as *Tetramorium caespitum* (Linnaeus, 1758), *Lasius niger* (Linnaeus 1758) and *Polyergus rufescens* (Latreille 1798) with *Formica rufibarbis* Fabricius, 1793 and *Formica cunicularia* Latreille, 1798 slaves (Fig. 1a). 40 metres further is the centre of the colony, exclusively consisting of the exits of the *L. neglectus* supercolony (Fig. 1b). The difference between the exit density of the two sampling sites (Fig. 1a,b) is also visible and proved by χ^2 test ($p<0.05$). We have also demonstrated that the Shannon diversity of the centre assemblage is significantly smaller than the diversity of the border ($p<0.05$).

The distribution of the exits is more equal than the individuals in traps and at the baits. There is a 50 metre long section (sampling points from 17 to 26) where *L. neglectus* existence and predominance is proved with all three sampling methods (Fig. 2a-c).

A relatively large number of *Lasius neglectus*, *L. niger* and *Tetramorium caespitum* were trapped (Fig. 2b). Occasionally *Formica rufibarbis*, *F. cunicularia*, *Tapinoma erraticum* and *Solenopsis fugax* workers were collected, too. It is also worth mentioning that on the base of maximal individual number from one soil trap the most abundant species was the *Lasius neglectus* (1719 individuals); *L. niger* follows it on second place with 219. The almost eight times larger maximal individual number of *L. neglectus* is thought-provoking.

Discussion

A characteristic feature of the strategy of the ants of *Lasius* subgenus is the less intraspecific aggression against individuals from other colonies, but they display a dominant position in the competitive hierarchy in the interspecific aggression and ascendancy in the number of individuals (Gallé 1980, 1985, 1994). The strategy of *L. neglectus* is similar in the centres of the colonies.

My previous unpublished results (Tartally 1999 2000) demonstrated the dominant position of *L. neglectus* in competitive hierarchy in many different habitats outcompeting all the other species in the centre of the supercolony.

According to the opinion of Gallé (1985, 1994), the existence of *L. niger* and similar species in the community raises the diversity to a limited level (plus one species), though afterwards the diversity will be reduced by the competitive exclusion.

On the edges of the colony the *L. neglectus* seems to avoid the meeting with other ant species. It ensues from this that interspecific aggression is less on the edge of the colony than in the centre. *L. neglectus* aggression may increase in colony centres, and the growing number of ant individuals also adds to this. *L. neglectus* rarely fell into the same soil traps with *L. niger* and/or *Tetramorium caespitum*. It shows that there are well defined colony and penetration area borders of these ants. The situation is different with *Formica rufibarbis* and *F. cunicularia*, because these two ant species sometimes have been found in the soil traps placed in the centre of the colony during my research. Unlike *L. niger* and *T. caespitum* the daily activity of *F. rufibarbis* and *F. cunicularia* are different from *L. neglectus* (Tartally 1999, 2000). This could be explained that these two species can venture far into the colony centres without meeting any *L. neglectus* individuals. However in Budatétény, these two species disappeared from the centre of the 2 km^2 sized *L. neglectus* supercolony (van Loon *et al.* 1990, Tartally 1999, 2000).

Long lifetime of polygynous supercolonies is their other advantage over monogynous species. In the centre of the colony there is no chance for other species' females to establish a colony successfully.

L. neglectus is probably an invasive, foreign fauna member in Hungary and it excludes other native species from colony centres. Due to its small size, the supercolony of the botanical garden doesn't really have a colony centre, so everything previously written are true to a greater extent for the supercolony in Budatétény which size is beyond 2 km^2. Presumably, *L. neglectus* also affect soil invertebrate communities, as it can exclude even *Paravespula* sp. from food sources (Tartally 1999, 2000).

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TISCIA 32, 47-54

COMPARATIVE ANALYSIS OF THE SMALL MAMMAL FAUNA OF THE RIVER DRAVA PLAIN REGION. I. SPECIES RICHNESS, DIVERSITY AND BIOMASS BASED ON THE ANALYSIS OF BARN OWL *TYTO ALBA* (SCOP., 1769) PELLETS

Gy. Horváth

Horváth, Gy. (2000): Comparative analysis of the small mammal fauna of the River Drava plain region. I. Species richness, diversity and biomass based on the analysis of Barn owl *Tyto alba* (Scop., 1769) pellets. — *Tiscia 32, 47-54.*

Abstract. A total of 2176 pellets and pellet fragments were analysed, all of which have been collected in 15 human settlements along the river Dráva, during 1996. From these 4335 small mammal specimens were identified. Among the Insectivora, six shrew species belonging to the family *Soricidae* and four insectivorous genera were identified, whereas among the rodents (Rodentia) 12 species were differentiated plus one genus-level (*Apodemus spp.*) identification was made. With the help of abundance data of the small mammal taxa identified from the pellets, a significant saturation curve was found to exist between sample size and number of species. With the increase of pellet number, the number of species did not change considerably. Shannon-diversity and evenness were not sensitive to sample size, but Margaleff's species richness values significantly decreased as the number of pellets increased. As a result of differences in sample sizes, the small mammal communities in only 9 of the settlements were compared using cluster analysis and variance analysis. I concluded from the results that several years of data collection is necessary to clearly describe the small mammal species composition of the studied region.

Keywords: small mammal diversity, species richness, biomass, pellet analysis, Tyto alba

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Introduction

Small mammals have an extremely important role in forming the structures of communities found in natural and seminatural habitats. They were significant objects of production biological research almost thirty years ago. Those studies are especially notable from the aspect of trophic structure analysis, energetics and biomass (Petrusewicz 1975, Petrusewicz and Hannsson 1975, Golley et al 1975). At the same time, other types of synbiological case studies were also produced, such as life history strategies (Gliwicz *et al.* 1968, Bujalska *et al.* 1968, Bujalska 1975, Flowerdew *et al.* 1985), questions of density estimation (Ryszkowski 1971, Smith *et al.* 1971, 1975), as well as the problems of temporal population changes, population regulation and cyclicity (Petrusewicz 1966, Chitty 1967, Krebs 1964).

In the framework of the National Biodiversity Monitoring System introduced recently in Hungary, special attention is focused on small mammal species (Csorba and Pecsenye 1997), since many of them are protected, are included in Red Data Books, and their populations have been thoroughly studied during the past 30 years. The alterations of their densities and community structures as a result of changes in the limiting background variables, indicate any decline in their environment (degradation, fragmentation, urban effects, pollution, etc.). In order to effectively conclude from the changes in small mammal populations and communities, it is necessary to perform synbiological investigation of populations and communities in several habitats or habitat complexes, to describe their spatio-temporal patterns, and analyse the changes in these patterns.

However, because the technical demands of sampling from small mammal populations and the investigation of their life characters (e. g. the use of various trapping techniques, radio telemetry) are pretty expensive, it is common to use indirect sampling, such as pellet analysis, in synphenobiological and ecological research. This method is not objectionable from a conservation aspect, and is a relatively fast way of collecting large amount of occurrence data. For the study of small mammal fauna (status survey, monitoring, diversity estimation) the most appropriate are the pellets of the barn owl (*Tyto alba*), because among the owl species occurring in Hungary this is the one with the widest selection of preys, and also the feeding ecology of this species is well studied (Kalivoda 1994, Csorba and Pecsenye 1997, Mátics 1997, Horváth and Jeney 1998). The relationship between small mammal populations and the feeding of the barn owl has been sought for from various aspects (Bohnsack 1966, de Brujin 1979, Kotler *et al.* 1988, Palotás 1979), and the connection of small mammal abundance and barn owl reproduction has also been studied (Bühler 1964). There has been collected also considerable amount of information on the energetics of the barn owl — small mammal relationship (Ceska 1980, Goszczynsky 1976, Kirkwood 1979). In Hungary, the representativity of pellet analysis as an indirect way of population sampling, and the problem of sample size have been studied mostly from a theoretical approach and based on literature data (Kalivoda 1993, 1994).

The reason the above issues are important from the point of view of indirect monitoring is the fact that owls can be selective: they can prefer some prey species to others. The question is that given such a prey preference and a switching of this attitude, to what extent the species composition appearing in the owl's food is representative of the small mammal fauna of the nearby areas, and samples collected in which period are best for the monitoring of small mammals (Horváth and Jeney 1998). Further, to what extent indices calculated from the basic data of pellet analysis (such as species richness, diversity, biomass) are applicable in following small mammal abundance data and in determining trends.

Several studies have been produced about barn owl pellet collecting and analysis (Fenyősi 1994, Horváth 1995, Purger 1998, Horváth 1998), which are faunistic summaries of various time intervals, with no attention to parameters derived from basic data and the relationships between them.

The aim of the present study is to analyse the relationships between derived community-ecological and feeding ecological characteristics obtained from the abundance data in pellets collected during a span of a year, and to compare the small mammal faunas of the sample areas.

Material and methods

Pellet samples and identification

The present study elaborates on the results of analysis of pellets collected in 15 human settlements along the river Dráva in 1996. Sampling, accordingly, covered the lower river section in Somogy county, and its entire section in Baranya county. The settlements of the collection sites were numbered from the east to the west. Thus the first sampling site is Tótokföldje (Old) in Baranya county, whereas the last one is Péterhida in Somogy county. Church towers as potential barn owl nesting sites were visited monthly from the beginning of the year, and were frequented as long as fresh pellets suggesting the presence of owls were found. There were no successful nestings in the belfries of the subject villages in 1996; in most of the cases fresh pellets found during the period between April-June originated from owls left without a pair. In the case of Péterhida, Szentborbás (2) and Tótokföldje, pellets were collected in abandoned farm buildings and old, uninhabited houses. The number of collections and the amount of the collected material (i. e. the number of pellets) varied among the 15 sites; these are summarized on a UTM-grid in the order of collection sites, in Table 1. The collected material included whole pellets as well as pellet fragments/debris in many cases. This is important to note because prey lists were compiled based on whole pellets only as well as on whole pellets plus pellet debris.

Taxonomic identification was done on the basis of skull characteristics and dentition (Schmidt 1967, Ács 1985, Ujhelyi 1994). The *Neomys* species (*Neomys fodiens* Pennant 1771, and *Neomys anomalus* Cabrera 1907) were differentiated by measuring the height of the corona-process of the mandible; if this was unfeasible, only the genus was identified (*Neomys sp.*). The wood mouse (*Apodemus sylvaticus* Linnaeus 1758), the yellow-necked wood mouse (*Apodemus flavicollis* Melchior 1834) and the pygmy field mouse (*Apodemus microps* Kratochvíl and Rosicky 1952) were categorized commonly as wood mice (*Apodemus spp.*) The house mouse (*Mus musculus* Linnaeus 1758) was differentiated from the gleaner mouse (*Mus spicilegus* Petényi 1882) on the basis of the length proportions of the upper and lower zygomatic arches (Demeter 1995, Demeter *et al.* 1995); when

these were missing from the skull or only a mandible was found, only the genus was indicated (*Mus sp.*).

Remains of both the rare ship rat (*Rattus rattus* Linaeus 1758) and the invasive house rat (*Rattus norvegicus* Berkenhout 1769) were discovered in the pellet samples. In cases when the skeletal material was insufficient for the exact differentiation of these two species, only the genus was indicated (*Rattus sp.*) Accordingly, evaluation was performed on the basis of a total of 23 small mammal taxa.

Statistical methods

When the collected material was summed up, only the whole pellets were brought into the analysis. The first important basic information was the number of pellets, a variable expressing the size of the sample and affecting its representativity. Thus, as a first step, regression analysis was applied for the relationship between number of pellets and number of species. Community indices were then calculated from the abundance data of samples with totalized pellet numbers, such as Shannon-Wiener diversity

$$H(S) = -\sum_{i=1}^{S} p_i \ln p_i,$$

and evenness

$$J = \frac{H}{\ln S},$$

where p_i is the proportion of the i-th species in the sample, H is diversity, and S is the number of species (Pielou 1975), and species richness based on Margaleff's index

$$d = \frac{S-1}{\ln N}$$

(Magurran 1988) where S is the number of species identified from the pellets, and N is the number of

Table 1. Pellet collection sites along river Dráva, with dates of collection, number of pellets and prey taxa

Site of sampling	UTM code	Yearly number of collection	Date of collection [1996]	Number of pellets / collection	Total number of pellets	Number of prey taxa
1. Tótokföldje (Old)	BR97	4	04. 01. 06. 20. 07. 27. 08. 31.	70 152 233 27	482	18
2. Gordisa	BR87	1	06. 20.	31	31	13
3. Matty	BR87	1	02. 24.	47	47	13
4. Kovácshida	BR87	5	02. 24. 03. 25. 04. 29. 06. 20. 09. 28.	6 21 4 36 2	67	10
5. Szaporca	BR77	5	02. 24. 03. 25. 04. 29. 05. 25. 06. 20.	14 3 89 8 2	116	15
6. Cún	BR77	5	02. 24. 03. 25. 04. 29. 05. 25.	67 67 55 4	193	19
7. Tésenfa	BR77	1	07. 27.	176	176	17
8. Kisszentmárton	BR67	4	02. 24. 04. 29. 05. 25. 06. 30.	48 39 11 11	109	17
9. Majláthpuszta	BR77	1	03. 25.	42	42	13
10. Vejti	YL37	3	02. 24. 03. 25. 04. 29.	26 15 15	56	16
11. Piskó	YL27	3	02. 24. 03. 25. 04. 29.	17 11 19	47	16
12. Zaláta	YL27	1	06. 29.	21	21	13
13. Drávasztára	YL17	1	06. 29.	8	8	5
14. Szentborbás(1.,2.)	YL08	1/1 2/1	09. 03. 09. 03.	6 48	54	13
15. Péterhida	XL98	1	07. 31.	79	79	18
Σ		61		2176	1528	216

individuals. Knowing the average body weight of the various species (von Knorre 1973, De Brujin 1979, Görner-Hackethal 1987, Ács 1985, März 1987), two feeding-ecological parameters were calculated from the samples with a given number of pellets, the following way:

- prey number in a pellet [*PN*]:

$$PN(specimens) = \frac{\text{number of prey items found}}{\text{number of pellets}}$$

- biomass eaten in a pellet [*BEP*]:

$$BEP(g) = \frac{\text{totalized body weight of prey items}}{\text{number of pellets}}$$

Then the relationships between the number of pellets and the three community characteristics (diversity, evenness, species richness), and between the two feeding-ecological parameters and the community characteristics were analysed using regression analysis.

Because sample sizes (number of pellets and number of collections) varied, the abundance values of various species in the different villages were standardized for 100 pellets:

$$\text{relative abundance} = \frac{\text{abundance} \times 100}{\text{number of pellets} \times \text{number of collections}}$$

where samples with fewer than 50 pellets were left out from the calculations. Based on these relative abundances, 9 settlements were compared using cluster analysis, where Chekanowski-index and the group average method were applied. Then the sampling sites were compared using the Kruskal-Wallis test of ANOVA. The program packages NuCoSA 1.05 (Tóthmérész 1993, 1996, 1997), BioDiversity (Lambshead *et al.* 1995), and Toxstat (Gulley *et al.* 1990) were used for these calculations.

Results

From the material collected in 1996 in 15 settlements along the river Dráva a total of 2176 pellets and pellet fragments were processed (Table 1.), and 4335 small mammal specimens were taxonomically identified. Among the Insectivora, six shrew species belonging to the family Soricidae and four insectivorous genera were identified, whereas among the rodents (Rodentia) 12 species were differentiated plus one genus-level (*Apodemus spp.*) identification was made. 1130 specimens belonged to the order Insectivora, out of which only one individual was a mole (*Talpa europaea* Linnaeus 1758), while the remaining were members of the Soricidae family.

Based on data from the whole pellets, a saturation curve (r = 0.8) was obtained for the correlation between number of pellets and number of prey taxa (Fig. 1). From this it appears that the number of species shows considerable variation up to a value of around 100 pellets beyond which the increase in the number of prey taxa is quite insignificant.

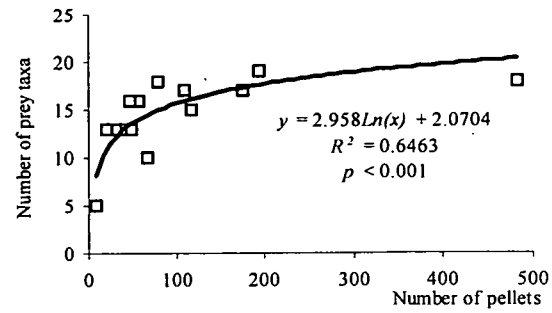

Fig. 1 Correlation between number of pellets and number of prey taxa, based on the entire sample.

When Shannon-diversity and evenness were plotted against the number of pellets, no significant correlations were found (Figs 2-3). The value of diversity can be quite variable up to 100 pellets, which is, of course, influenced not only by lower or higher values of the number of species, but it is also influential whether or not the barn owl prefers certain preys to others, because the higher relative frequency of a preferred prey animal reduces the diversity of its sample. Evenness is less variable in this range of pellet number. A sample of low number of pellets, collected once in a given period provides little information about the prey choice of an area and about the hunting strategies of the owls. One reason of the greater evenness values may be that the frequencies of the few specimens identified from a small sample are quite similar, and another possibility is that in spring when the density of the common vole (*Microtus arvalis* Pallas 1779) is still low, barn owls feed on a greater variety of prey which fact results in an increased evenness of the sample.

In the case of samples with low pellet numbers, species richness values are generally much higher than at greater pellet numbers. The value of species richness shows significant exponential decrease as the number of pellets grow (r = 0.947). Margaleff's index of species richness is much more sensitive to the number of pellets determining sample size, than diversity or evenness. Its main reason is that in the species richness formula abundance appears in the denominator, and because abundance grows linearly

with the number of pellets, species richness values calculated from a sample of few pellets containing less specimens will be much lower than with a sample of high number of pellets.

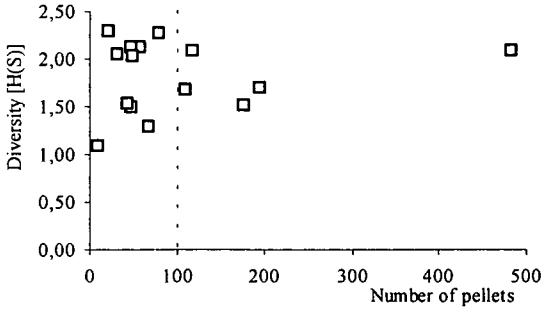

Fig. 2 Shannon-diversity as a function of number of pellets.

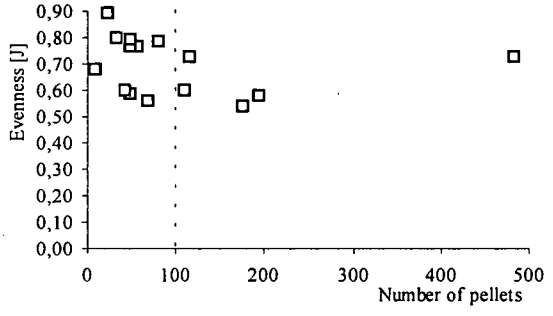

Fig. 3 Evenness as a function of number of pellets.

No significant correlation was found when BEP and PN were tested for relatedness with the community characteristics. The values of the two feeding-ecological indices show moderate variation, since these are indices that level off differences in sample sizes, thus in a comparison with the community parameters, there is no mathematically describable significant correlation between the analysed variable-pairs.

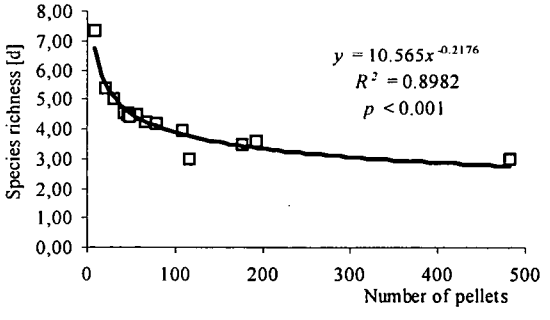

Fig. 4 Correlation between number of pellets and Margaleff-index.

Collection sites with samples larger than 50 pellets were compared based on their relative abundances. The cluster analysis separated two main groups between which there is a relatively great distance (Fig. 5). The smaller cluster on the right is more uniform, with the two villages in Somogy county clearly separated and with the species composition of only one sampling site in Baranya county showing similarity to them. In the other cluster there are samples from county Baranya only, but this one is not as uniform as the other. The small mammal composition obtained for Vejti (7) is especially different from the rest of the collecting sites in Baranya.

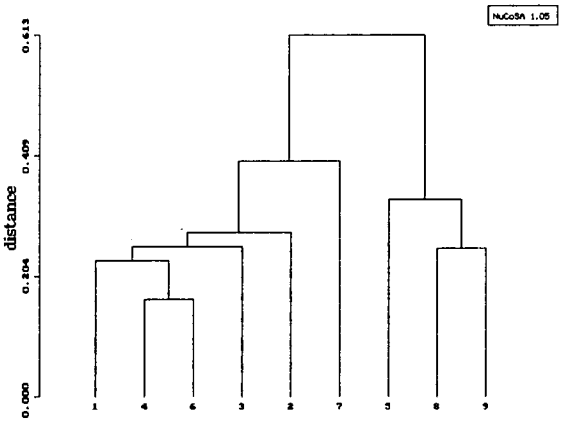

Fig. 5 Comparison of the small mammal communities found in the selected 9 settlements, using cluster analysis and Chekanowski-index. 1: Tótokföldje, 2: Kovácshida, 3: Szaporca, 4: Cún, 5: Tésenfa, 6: Kisszentmárton, 7: Vejti, 8: Szentborbás, 9. Péterhida .

The variance analysis of relative abundance values did not reveal significant difference between any of the sampling sites (H = 12.125, *NS*) from which result it seems likely that in order to be able to demonstrate any slighter difference between the small mammal communities of the various areas much larger amount of data is necessary, which can be obtained only by means of a long-term monitoring program.

Discussion

As part of the National Biodiversity Monitoring System, small mammal monitoring based on country-wide owl pellet collection is planned to be introduced in the form of a separate sub-project. Therefore it would be extremely important to find out how the samples collected in a given period but with varying pellet numbers should be standardized, and from the abundance data of the identified prey

species what sort of derived data are applicable for the comparison of samples collected in different times and at different locations. The present paper elaborates on one year's data of a region. From the experience gained during the collections in that year it appeared that it was extremely difficult to fulfill the regularity requirement of monitoring in the study area which follows the course of river Dráva, and to reach every nesting site within a pre-set time interval. The amount of pellets that could be collected regularly from a given nesting site was strongly influenced by how the birds used the site, i. e. whether the church tower was used only as a resting site, a pairless owl occupied the building, or a successful nesting (perhaps a second nesting, too) could be recorded at that place. For this reason the number and size of the samples collected at the 15 villages showed great variation. Based on our results it appeared that the number of species identified from the pellets did not grow further when the number of pellets reached 100 in a sample. Kalivoda (1994), when dealing with the problems of sample size and representativity, concluded that sample sizes of around 200 were appropriate, and even with samples much greater than that only very limited increase could be expected in the number of species. Based on his results, sample sizes of 50 to 100 quite well approximate the number of taxa obtained from greater samples.

From among the three community characteristics, diversity and evenness appeared to be more applicable derived data, for their values were not affected considerably by sample size. However, Margaleff's species richness index proved to be very sensitive to sample size; it is not advisable to calculate it in the case of samples with highly varying number of pellets. PN and especially BEP are appropriate indices for describing feeding rhythms. The values of BEP are determined by the entire food base as a whole, it is not sensitive to the relative frequencies of the various species. It is well in line with the population growth of small mammals in autumn, and it is affected also by the nesting time and nesting rhythm of the owls.

Our regular pellet collecting along river Dráva provides a general idea of the composition of the small mammal faunas around the sampling sites, even after only one year. It is a contribution to the knowledge about the fauna of the southern part of Baranya county along river Dráva with important distribution data, which supplements earlier, sparse data from this county (Schmidt 1969, 1972, 1974, 1975), as well as more detailed surveys having done here (Horváth 1994, 1995, 1998). The first finding to be noted among the processed data is the list of the frequent species. If a comparison is made with results obtained on the Dráva lowland between 1985 and 1994 (Horváth 1995), it appears that at present the most frequent *Microtus* genus is not followed by *Sorex*, but instead the next most frequent species are *Crocidura* and *Apodemus*. This change may be related with the fact that pellet collection sites of those investigations and the present study only partly overlap. Because of the variation of sample sizes, small mammal communities of only 9 of the 15 villages were compared. Relative abundance, a parameter corrected for differences in sample sizes and abundance, was used as input for the cluster analysis, which proved to be appropriate for performing the multivariate statistical procedure. As a surprising result, the two settlements in Somogy county appeared to be in considerable separation, with Tésenfa, a village in Baranya being most similar to them. However, conclusions can only be made with precaution, because variance analysis did not indicate any significant difference. Therefore it remains a question how notable (if any) statistically provable difference can be expected between the small mammal communities recorded in an indirect way of sampling on the spatial scale of areas along river Dráva. Can the variation in climate, terrain and vegetation occurring along this scale cause differences in the composition of small mammal faunas? To what degree the demonstrable results are determined by food preference and density-dependent predation in the owls? In order to be able to answer these questions a successful monitoring and the analysis of data from a number of years are necessary.

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TISCIA 32, 55-60

METAL POLLUTION INDEX: PROPOSAL FOR FRESHWATER MONITORING BASED ON TRACE METAL ACCUMULATION IN FISH

I. Teodorovic, N. Djukic, S. Maletin, B. Miljanovic and N. Jugovac

Teodorovic, I., Djukic, N., Maletin, S., Miljanovic, B. and Jugovac, N. (2000): Metal pollution index: proposal for freshwater monitoring based on trace metal accumulation in fish — *Tiscia 32,55-60.*

Abstract. Different fish species (benthivore and piscivore) from the Channel System Danube - Tisza - Danube in Yugoslavia have been analysed for body trace metal burden with an aim to establish freshwater metal pollution biomonitoring. New Metal Pollution Index (MPI) which distinguishes polluted from unpolluted ecosystems is proposed, based on acquired knowledge on metal bioavailability, bioconcentration and bioaccumulation patterns. This simple mathematical model is calculated upon logarithmically transformed values of metal concentrations in fish tissue, in order to achieve normal distribution of the element values and to diminish the more than thousand-fold differences between the least and the most abundant elements. MPI is the simplest way which enables presentation of all results from the metal concentrations as one value, by using a normalizer (reference values for lower part of the Danube Basin) to account for biological variation in non-polluted areas. Since no biomagnification within fish trophic chain has been observed, benthic fish seem to be, due to the highest metal burden, more suitable for evaluation. Furthermore, *Carassius auratus gibelio*, being presently one of the most abundant species inhabiting the Yugoslav part of the Danube watershed is being proposed as sentinel organism.

Key words: trace metals, Metal Pollution Index, freshwater monitoring, fish, Danube Basin

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Introduction

International waters, particularly large watersheds as the Danube river basin is, are subjected to both huge amounts of waste water input and different legislative and enforcement measures. Therefore, there is a need to establish internationally standardised methods for freshwater monitoring, which seems not to be a problem when considering chemical analysis of sediments, waters and wastewaters. However, biological monitoring (using bioindicators) is highly dependent on biodiversity and species richness within the region. Therefore, the objective of this paper is to contribute to finding the most appropriate model and applicable sentinel organisms for trace metal pollution monitoring within the lower part of the Danube river basin.

Trace metal accumulation patterns in aquatic biota, along with bioconcentration and biomagnification processes have been excessively studied in the Danube Basin (Salanki *et al.* 1982, Pujin *et al.* 1990, Wachs 1991, Maletin *et al.* 1992). Some valuable attempts have been made (Wachs 1992/93) in introducing the classifications of freshwater ecosystems according to fish muscle metal burden. Such evaluations have even been applied for the Yugoslav part of the Danube watershed (Maletin *et al.* 1996, Djukic *et al.* 1998a), with an idea that fish muscle metal burden could be more reliable water quality indicator than chemical analysis of water-column and sediment. Fish tissue metal content has also been successfully used in estimations of trace metal input into large European and American rivers. (Allen-Gil and Martynov 1995, Chevreuil *et al.*

1995, Saiki *et al.* 1995, Carru *et al.* 1996). However, the cited literature reviled extremely diverse approaches to choosing the most appropriate fish group, species and tissue for these kind of monitoring studies. Considering that fish tissue preparation methods and analytical methods for metal determinations as well have been standardised so far (APHA 1989, U.S. EPA 1991), the attempt herein has been made in finding the most suitable species and tissues for bioaccumulation studies and particularly the simplest mathematical model for evaluation and comparison of results, at least within the region.

Material and methods

The fish for this study was caught in spring 1997, in irrigation channel Danube - Tisza - Danube at the dam in Becej (separating Tisza from the channel) and in microreservoir "Moharac" which, due to its position (remote from industry, urban zones and major roads) served as reference site. The sample consisted of piscivore - *Silurus glanis* (from both locations), *Tinca tinca* (from the channel) and *Carassius auratus gibelio* and *Abramis brama* (from the microreservoir). Investigated tissues included muscle, gills, liver and kidney. Tissue digestion and sample preparation was done according to standard procedure (U.S. EPA 1991) and Perkin Elmer AAS (flame and graphite furnace with background correction) was used for Cd, Zn, Cu, NI, Cr, Pb and Al determinations (APHA 1989). All results are presented on a wet weight basis, as mg/kg, but recalculation factors from wet to dry weight basis are presented for comparative purposes: muscle 5; liver 2.6 and kidney 5.5. Means ± SD are chosen in the presentation of data, statistical significance was assigned at $p \leq 0.05$ after one way ANOVA analysis. In all statistical analyses, values bellow the detection limits were replaced by half of the detection limit. Fish muscle metal burden has been used for environmental evaluation (Teodorovic *et al.* 1998). Metal Pollution Index (MPI) has been introduced and calculated for the chosen site, yet the calculation will be explained further in the text.

Results and Discussion

Three species from reference site (microreservoir "Moharac") with total number of 25 specimens, and two species from the chosen site of the Danube - Tisza - Danube (Becej dam) Channel with 20 specimens in total, were analysed on metal body burden. As the literature revealed very few differences in heavy metal concentrations between sexes (Scharenberg *et al.* 1994), this parameter was not included in the data handling. The available data (number of samples) are too few to allow the analysis of age/size/weight dependence. However, grand scale studies (Strip *et al.* 1990, Spry et Wiener 1991, Dietz *et al.* 1996) showed that concentrations of metals within a fish population (with exemption of Hg) do not typically increase with increasing age or body size. Consequently, these parameters were also omitted in the data handling.

The results presented in Figures 1-5 show tissue/organ distribution pattern of metal accumulation in wels. Generally, the patterns in other species are quite similar, although the sites for accumulation vary with route of uptake and are, to a certain extent, species-specific. The highest concentrations of Cd (0.82 mg/kg wet wt.) were detected in kidneys, then in liver (0.19 mg/kg. wet wt.) which is in accordance with previous findings (Kraal *et al.* 1995, Allen 1995, Djukic *et al.* 1998b). Cu, Pb, and Zn accumu-

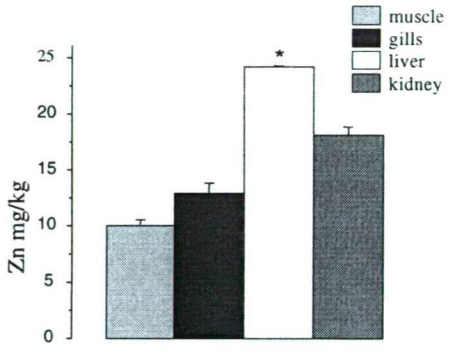

Fig. 1 Distribution of Zn in the tissues of wels from DTD Hydrosystem (sampling site Becej dam). Values represent mean±SD (n=10); * -significant difference $p \leq 0.05$

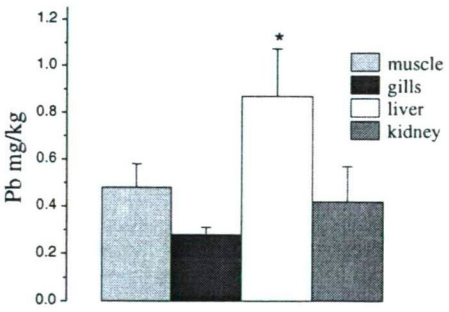

Fig. 2 Distribution of Pb in the tissues of wels from DTD Hydrosystem (for legends see Fig. 1)

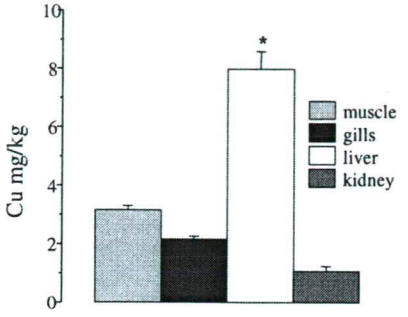

Fig. 3 Distribution of Cu in the tissues of wels from DTD Hydrosystem (for legends see Fig. 1)

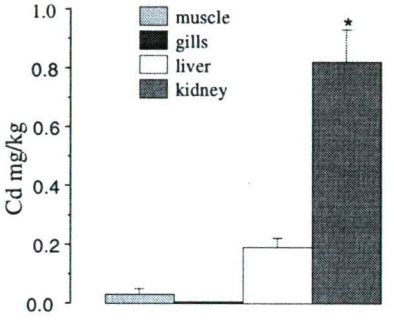

Fig. 4 Distribution of Cd in the tissues of wels from DTD Hydrosystem (for legends see Fig. 1)

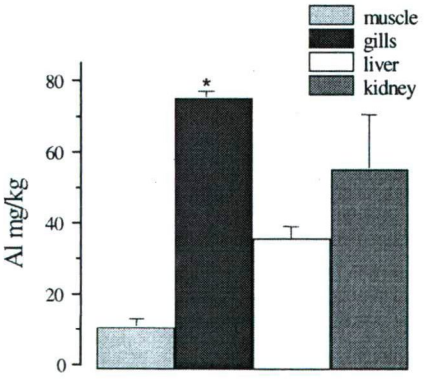

Fig. 5 Distribution of Al in the tissues of wels from DTD Hydrosystem (for legends see Fig. 1)

lated chiefly in liver, (8.01, 0.87, and 24.2 mg/kg wet wt. respectively) which is also reported to be the pattern. (Salanki *et al.* 1982, Pujin *et al.* 1990, Jorgensen and Pedersen 1994).

The highest Al concentrations were found in gills (76 mg/kg wet wt.) and these findings support the existing theory (Poleo *et al.* 1997) that fish exposed to aqueous Al (both in laboratory and field) readily accumulate the metal in and on the gill, and that concentrations of the analyte are much less in blood and internal organs. According to our results, muscle proved to be the tissue with low metal burden of all analytes, which supports the thesis that metal contamination of fish, particularly with cadmium and lead, generally need not to be considered as a significant health risk to human consumers (Spry and Wiener 1991).

Comparison of muscle metal burden in piscivore (wels) and benthivore (tench) is shown in Fig. 6 Zn, Cd, Al and Ni content was slightly while Pb concentration was significantly higher in tench.

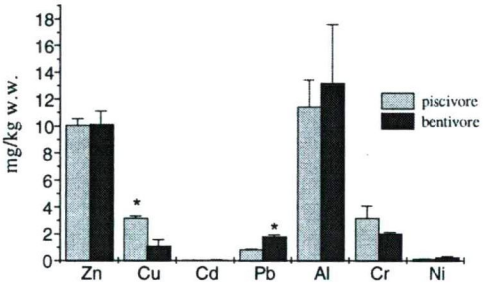

Fig. 6 Muscle metal burden in piscivore (wels) and benthivore (tench) from the DTD Channel. Values represent mean±SD (n=10 for piscivore and 15 for benthivore); * -significant difference $p \leq 0.05$

Essential micronutrients Cu and Cr were detected in higher concentrations in wels, which could be explained by species-specific requests and regulation of these elements. These results lead to a conclusion, which stands in accordance with previously published data (Scharenberg *et al.* 1994, Carru *et al.* 1996, Djukic *et al.* 1998b), that no biomagnification within the fish trophic chain could be observed Consequently, benthivore fish muscle metal content has been used for environmental evaluation.

According to Wachs (1991, 1992/1993), metal concentrations in freshwater fish muscle could be used in hydroecosystem classification. Consequently, the cited author introduces an environmental classification of freshwater ecosystems into 7 class-

es, based on muscle metal burden of the Danube fish, with no specification of species. In previously published papers (Djukic *et al.* 1998c, Teodorovic *et al.* 1998) we slightly revised the mentioned classification and suggested a new one, more suitable for the lower part of the Danube. Moreover, our opinion is that this newly proposed classification of freshwater ecosystems (also based on fish muscle metal content) more precisely takes into account standard detection limits for chosen metals, influence of local/regional geochemistry and food standard limits within the region. Basically, detection limits (APHA, 1989, US EPA, 1990) are set as an ambient standard for I class waters. Ranges for I-II class waters are set to cover minimal detected Cu, Cr and Zn concentrations (0.33. 0.1 and 5 mg/kg wet wt., respectively) in fish muscle from unpolluted reference site (Moharac microreservoirs). (Teodorovic *et al.* 1998). These values represent the lowest concentrations measured after analysing more than 20 locations within the Yugoslav part of the Danube basin. (Pujin *et al.* 1990, Maletin *et al.* 1992, Maletin *et al.* 1996, Djukic *et al.* 1998a, b, c, Teodorovic 1997, Teodorovic *et al.* 1998). Therefore, microreservoir Moharac has been chosen as a reference site to provide information on natural metal levels in non-contaminated fish, from non-polluted areas located within Yugoslav (e.g. lower part of the Danube River Basin). National food standard limits for edible fish tissues (Cd - 0.1 mg/kg wet wt. and Pb - 1 mg/kg wet wt. - Anon. 1992) are set as upper ambient standard values for III class waters.

Table 1, therefore, represents the environmental evaluation of Danube - Tisza - Danube Channel (sampling site Becej) based on tench muscle metal burden. Such classification could be useful in human health risk evaluation, providing it deals only with edible fish tissue metal load. It should be pointed out that Pb concentration in tench from the mentioned location exceeds Yugoslav food limits for edible fish tissue (1 mg/kg wet wt).

However, there are many problems with implementation of this classification. First of all, in total score, it treats highly toxic (Cd, Pb) and essential metals (Zn, Cu) equally. Further on, it doesn't represent the actual state of the chosen ecosystem as it is made on muscle burden basis, although it has been showed that other tissues (liver, kidneys and gills) could be basically refereed to as "target zones". (Figs 1-5). Moreover, it doesn't calculate for biological variability, since the exact sentinel species hasn't been chosen.

Therefore, we made an attempt in introducing Metal Pollution Index (MPI) as a mathematical model which could solve some of the highlighted problems. MPI has been calculated to enable presentation of all results from the metal concentrations (Cd, Cu, Zn, Pb and Al) as one value if possible, yet overcoming the difficulties with both application and understanding of demanding statistical analysis. According to Jorgensen and Pedersen (1994), this implies that the five metal concentrations must be normalised to make it possible to sum up and average the different metal concentrations into one value. We have chosen the average values of fish tissue burden (liver for Cu, Zn and Pb, gills for Al and kidney for Cd) from the reference site. (Moharac microreservoir) Such normaliser is used to account for the biological variation in a non-polluted area. Since no significant difference has been found between metal concentration in wels, prussian carp and bream from the reference site, (Teodorovic 1997, Teodorovic 1998 - unpublished) the sample has been pooled so the reference values represent the mean of 25 specimens. (Table 2). Furthermore, the data were logarithmically transformed to achieve normal

Table 1. Ecosystem classification based on fish muscle metal burden and environmental evaluation of DTD channel (at Becej dam)

class	Cd	Cu	Ni	Cr	Pb	Zn	
I	<0.015	<0.3	<0.03	<0.1	<0.01.	<5	
I-II	0.015-0.02	0.3-0.5	0.03-0.1	0.1-0.5	0.01-0.05	5-10	
II	0.02-0.05	0.5-1	0.1-0.4	0.5-1	0.05-0.1	10-15	
II-III	0.05-0.08	1-2	0.4-1	1-1.5	0.1-0.5	15-20	
III	0.08-0.1	2-3	1-2.5	1.5-3	0.5-1	20-25	
III-IV	0.1-0.5	3-5	2.5-4	3-5	1-2	25-35	
IV	>0.5	>5	>4	>5	>2	>35	
muscle content tench mean±SD n=10	0.02±0.005	1.07±0.5	0.23±0.1	1.99±0.1	1.78±0.12	10.14±1	TOTAL
class	I-II	II-III	II	III	III-IV	II	II-III

Table 2. Metal Concentrations in wels and tench tissues; Reference values and MPI

	tissue	wels mg/kg wet wt. Mean (n=10) ±SD	(x) - tench mg/kg wet wt mean (n=10) ±SD	reference value (ref.)	x/ref.
Zn	liver	24.2± 1	34 ± 9*	20	1.7
Cu	liver	8 ± 0.6	13.7 ± 2.5*	2	6.85
Pb	liver	0.87 ± 0.3	3 ± 0.6*	0.2	15
Cd	kidney	0.82 ± 0.3	0.9 ± 0.2	0.1	9
Al	gills	76 ± 1	85 ± 3*	20	4.25
				Σ	36.8
			MPI=	log Σ	1.57

*p≤0.05 - significantly different from wels; reference values mean (n=25) concentrations in pooled sample from Moharac reservoir

distribution of the element values and, what is more important, to diminish the more than thousand-fold difference between the least and the most abundant elements. Without such transformation, the least abundant elements would be without influence on the results. (Julshamn and Grahl-Nielsen 1996).

MPI has been calculated as:

$$MPI = \log \sum_{i=1}^{n=5} \frac{[\bar{x}]}{ref_i}$$

where ref_i represents a normalizer, or a reference value for each of five chosen metals (Cd, Cu, Pb, Zn and Al) in selected tissues, while x represents mean value (n≥10, SD up to 30%) of metal concentration in the same tissues from the chosen sampling site. If calculated as proposed, MPI distinguishes „polluted" from „non-polluted" ecosystem: if this combined index is above 1 the concentrations of trace metals would be considered elevated and ecosystem could be regarded as „polluted".

Table 2 presents the liver, kidney and gill concentrations of Zn, Cu, Pb, Cd and Al in wels and tench from Channel Danube — Tisza — Danube (location Becej) and the reference values from unpolluted site. As all metal concentration in selected tissues were higher in tench than in wels (all but for Cd significantly), tench metal load has been used in MPI calculation. Also, the values of x/ref. ratio are presented and MPI is calculated. According to its value (1.57) location Becej could be regarded as „polluted" when trace metals are concerned. As it is obvious from the Table 2, the main component of the index is the Pb concentration, followed by Cd. The rest of the concentrations (essential and in the same time the most abundant metals) tend to contribute to a smaller extent. Our opinion is that this is the point where the muscle metal burden - based classification failed, while MPI succeeded: the most toxic and hazardous elements (Pb and Cd) contribute the most to this combined index.

Although fish do not fulfil all requirements for indicator organism (e.g., they are not sedentary) OECD and ICES agreed upon using trace metal concentrations in stationary fish as possible indicators in areas affected by human activities. (Jorgensen and Pedersen 1994). As benthivore fish proved to accumulate higher amounts of trace metals than piscivores, our opinion is that adequate sentinel species could be chosen from this group. To avoid possible species-specific differences, our suggestion is that *Carassius auratus gibelio*, which, according to recently published data (Jankovic 1994, Maletin *et al.* 1997) makes up to 50% out of total catch in Yugoslav part of the Danube Basin, could successfully serve as sentinel species. Besides, to diminish possible age/size influence on trace metal content, our suggestion is that only specimens belonging to same age group must be used in MPI calculations. Moreover, as seasonal variations in metal content has been observed (Balogh *et al.* 1985, Kock *et al.* 1996), the sampling has to be undertaken always within the same season, at precisely the same location. Our opinion is that MPI, calculated as proposed, with all applied constrains, could serve, in future, for time trend analysis of metal pollution within the region. In spite of indisputable importance of established chemical, biochemical and biological methods, our stingiest belief is that MPI might be included in complex freshwater monitoring programmes since it could produce some additional information on metal bioavailability, bioconcentration and metal input into the environment.

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